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IN THE U.S. PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF APPEALS AND INTERFERENCES

Inventor **Gabor BOGYE**

Patent App. **09/890,029**

Filed **24 July 2001**

Conf. No. **6045**

For **PHARMACEUTICAL COMBINATION OF PROGESTERONE
AND FOLIC ACID**

Art Unit **1617**

Examiner **Hui, S**

Hon. Commissioner of Patents
Box 1451
Alexandria, VA 22313-1451

Appealed 29-Sept-06

APPEAL BRIEF UNDER 37 CFR 41.37

Now comes appellant by his duly authorized attorney and submits his brief under the provisions of 37 CFR 41.37.

(i) REAL PARTY IN INTEREST

The real party in interest is the Appellant Gabor Bogye.

(ii) RELATED APPEALS AND INTERFERENCES

There are no prior or pending appeals, interferences or judicial proceedings known to Appellant or to the Appellant's legal representative, which may be related to, directly affect, or be directly affected by or have a bearing on the Board's decision in the pending appeal.

Appellant notes that long prior to the filing of this appeal on 29 September 2005,

Appellant had already filed a Notice of Appeal and already paid the fee of \$250 using Form PTO 2038. Appellant followed up on 19 July 2005 by filing an Appeal Brief Under 37 CFR 41.37 and paid the fee of \$250 again using Form PTO-2038. Upon receiving Appellants's appeal brief the Examiner on 11 October 2005 reopened prosecution of this application and gave Appellant a new office action. In the new office action the Examiner indicated that should Appellant in the future initiate a new appeal, both the Appellant's original payment for filing the notice of appeal and the original payment for filing the appeal brief may be applied to the new, present appeal. Thus no filing fee need accompany the filing of this appeal brief.

(iii) STATUS OF CLAIMS

Claims 1 through 8, 14, 17 and 18 have been canceled.

Claims 9 through 12, 20, and 25 through 39 have been rejected.

(iv) STATUS OF AMENDMENTS

Appellant filed a Response Under 37 CFR 1,116 After Final Rejection on 4 August 2006 in which no changes were made in the claims, but in which Appellant re-argued for the patentability of all pending claims over the prior art of record. In addition Appellant made of record a number of prior art references in support of his argument that claims 20, 26 through 28, 32, 33, 34, 35 and 36 were clear and definite and in full compliance with the requirements of 35 USC 112, second paragraph. Appellant also reargued that all of the claims in the application were free of the prior art and should not be rejected under either 35 USC 102 as anticipated or under 35 USC 103 as obvious. Appellant received an Advisory Action mailed by the Examiner on 3 October 2006 indicating that the Response Under 37 CFR 1.116 had been considered, but that there were no changes in the rejection of the claims under either 35 USC 112, second paragraph, 35 USC 102 or 35 USC 103.

(v) Summary of Claimed Subject Matter

A first feature of the invention is a method of treating a patient undergoing treatment with a gestagen hormone composition for hormone replacement therapy, for inflammation, for an in vitro fertilization program, for dermatological therapy or for cosmetological treatment to reduce a risk to the patient of thromboembolism induced by taking the gestagen hormone, which comprises the step of administering to the patient simultaneously, previously or subsequently to taking the gestagen hormone composition a therapeutically effective amount of a plasma homocysteine reducing agent. Antecedent basis for this aspect of the invention may be found in the specification on page 3, line 28 through page 6, line 32.

A second feature of the invention is a method of reducing a risk to an otherwise healthy patient of thromboembolism induced by administration of a gestagen hormone to said patient comprising the step of administering to the patient simultaneously, previously or subsequently to taking the gestagen hormone composition a therapeutically effective amount of a plasma homocysteine reducing agent. Antecedent basis for this aspect of the invention may be found in the specification on page 3, line 28 through page 6, line 32, and the first line of Examples a, b, and c on pages 7 and 8.

A third feature of the invention is a method of treating a patient taking a composition comprising a gestagen hormone to reduce a risk of thromboembolism induced by taking the gestagen hormone, which comprises the step of administering to the patient simultaneously, previously or subsequently to taking the composition comprising the gestagen hormone, a therapeutically effective amount of Vitamin B₁₂, betaine, choline or acetyl cysteine. Antecedent basis for this aspect of the invention may be found in the specification on page 3, line 28 through page 6, line 32.

A fourth feature of the invention is a method of reducing a risk to an otherwise healthy patient of thromboembolism induced by administration of a gestagen hormone composition to said patient comprising the step of administering to the patient simultaneously,

previously or subsequently to taking the gestagen hormone composition a therapeutically effective amount of a plasma homocysteine reducing agent other than folic acid. Antecedent basis for this aspect of the invention may be found in the specification on page 3, line 28 through page 6, line 32, and Examples a, b, and c especially, page 7, lines 4 and 19, and page 8, line 3.

A fifth feature of the invention is a method of reducing a risk to an otherwise healthy patient who may be threatened by an elevated plasma homocysteine level as a result of the administration of the gestagen hormone composition which comprises the step of administering to the patient simultaneously, previously or subsequently to the gestagen hormone, a therapeutically effective amount of a plasma homocysteine reducing agent. Antecedent basis for this aspect of the invention may be found in the specification on page 3, lines 28 to the bottom, pages 4 through 6 and in the specific examples.

(vi) Grounds for Rejection to be Reviewed on Appeal

The following issues are to be determined in this appeal:

1. Whether claims 20, 26 through 28, 32 through 35 and 39 should be rejected under 35 USC 112, second paragraph, as vague and indefinite;
2. Whether claims 9, 11, 20, 27, 32, 33, 34, 35 and 36 should be rejected as anticipated under 35 USC 102e or as obvious under 35 USC 103 in view of US Patent 6,190,693 to KAFRISSEN et al;
3. Whether KAFRISSEN et al is an effective prior art reference against any claim on appeal in view of Appellant's submission of a Declaration Under 37 CFR 1.131 during the prosecution of this application;
4. Whether claims 20, 28, 29, 31, 32, 33, 35, 37 and 39 should be rejected under 35 USC 102 as anticipated or under 35 USC 103 as obvious in view of SPELLACY et al;
5. Whether claims 20, 27, 32, 33, 34, 35 and 39 should be rejected under 35 USC 102 as anticipated or obvious under 35 USC 103, in view of BUTTERWORTH et al; and

6. Whether claims 9 through 12, 20 and 25 through 39 should be rejected under 35 USC 103(a) as unpatentable over US Patent 5,654,011 to JACKSON in view of FERMO et al.

(vii) The Arguments

Claims 20, 26 through 28, 32 through 35, and 39 should not be rejected under 35 USC 112, Second Paragraph

The Examiner has finally rejected claims 20, 26 through 28, 32 through 35, and 39 under 35 USC 112, second paragraph, as vague and indefinite. The Examiner maintains that claim 39 recites the limitation "for contraception" in line 3 and that there is improper antecedent basis in the claim for this limitation. Appellant disagrees since there is no definite article that is used in front of the expression "for contraception." Furthermore the fact that Appellant deleted in the previous amendment the term "for contraception" from claim 20 does not mean that Appellant is required to maintain this expression in claim 20 to provide antecedent basis for the term in claim 39.

The Examiner further maintains that it is not clear as to who is a healthy patient and whether a patient who suffers side effects such as headache or depression after taking a gestagen hormone is a healthy patient. Appellant disputes that these claims are vague and indefinite.

The term "otherwise healthy patient" in the case of treatment with hormone for contraception defines a patient who is healthy at the beginning of the hormone treatment and remains healthy in the course of the whole treatment. The term "otherwise healthy patient" in indications other than contraception means that apart from the indication of the hormone therapy the patient is healthy at the beginning of the hormone treatment and remains healthy in the course of the whole treatment.

The fact that the patients according to the presently claimed invention remain

healthy during the course of the whole treatment is supported by the following:

- The clinical trials disclosed in the present specification are qualified as Phase I trials, which, according to the professional rules, have to be suspended if clinically unexpected effects emerge;
- Also according to the professional rules, any such unexpected effect has to be reported in the summary.

Accordingly, the fact that the Appellant could complete his examinations evidences that his patients remained healthy in the course of the whole treatment. On page 8 in lines 20-21 it is specifically mentioned that "no undesired pregnancy or thromboembolic complication occurred". If any unexpected effect had occurred, it would have been mentioned in the specification.

The term "otherwise healthy patient" is used throughout the relevant technical literature, including US Patents, to define particular patients receiving any number of therapies. For instance in the FERMO et al reference cited by the Examiner, page 1, paragraph 5, and page 3, paragraph 2, describes the test patients as "healthy persons" without any further explanation which indicates that the meaning of the term is well understood by those skilled in the art. Furthermore see the enclosed literature abstracts: J. Clin. Ultrasound 2005, Feb; 33(2): 63 to 66 which mentions "healthy pregnant women" as patients; Dev Med Child Neurol. 1990 Dec; 32(12) 1058 to 1060 which defines the patients as "normal children." See also Eur. J. Echocardiogr. 2005, Jul. 18 which describes treatment of a "healthy population" of patients.

In addition see claim 1 of US Patent 6,562,790 which is directed to a method for abating coronary artery blockage in otherwise healthy male and female human subjects and claim 1 of US Patent 5,855,920 which is directed to a hormone replacement method comprising measuring hormone levels in a sample of an otherwise healthy human's subject blood. Accordingly there is nothing that is at all vague and indefinite about the term "an otherwise healthy patient" used in several of the claims now presented.

Claims 9, 11, 20, 27, 32, 33, 34, 35, and 36 should not be rejected as anticipated under 35 USC 102 or as obvious under 35 USC 103 in view of KAFRISSEN et al.

Appellant's further remarks serve to both establish that the claims on appeal are clear and definite in using the term – otherwise healthy patient - and that claims 9, 11, 20, 27, 32 through 36 and 39 are patentably distinguishable over the KAFRISSEN et al reference and so no rejection of any claim should be maintained as anticipated under 35 USC 102 or as obvious under 35 USC 103 in view of KAFRISSEN et al.

The method of KAFRISSEN et al relates to patients who are afflicted with or predisposed to become afflicted with a disorder due to which they have to be steadily treated with folic acid. This means that the patients of KAFRISSEN et al have to be treated with folic acid independently of the hormone treatment. The present invention, however, relates to healthy patients who do not need folic acid treatment unless they take a gestagen hormone.

In other words, KAFRISSEN et al relates to patients for whom the incidence of certain disorders is higher than normal, while the same for the patients of the invention is non-higher than normal, i.e. normal.

The Examiner argues that an individual having a risk factor can be considered as healthy. Appellant does not agree with this opinion. Although it is agreed that "having the risk factors of a disease is not equal to having the disease itself, but, according to the Appellant, having the risk factors of a disease is not equal to being healthy. Accordingly, individuals can be sorted into three groups: healthy individuals, individuals having the risk factors of a disease, and ill individuals.

In addition, Appellant does not agree with the opinion of the Examiner (last paragraph, page 2), according to which "It is not clear what patients or individuals would be considered "healthy"..., as the term "healthy" is a well-defined one. The following several references cited provide evidence that the term "healthy" /"otherwise healthy" is clear and unambiguous for one skilled in the art and so the rejection of the claims

under the second paragraph of 35 USC 112, should not be maintained:

According to the definition given in Adipex Phentermine Diet Pills, Diet Pills Glossary of Weight Loss Terms, (2002) appearing on the internet at <http://www.adipex-phentermine-dietpills.com/diet-pills-glossary.asp>), "Health is defined as - The overall condition of an organism at a given time in regard to soundness of body or mind and freedom from disease or abnormality."

According to this definition, neither individuals with diseases nor individuals with other abnormalities (e.g. laboratorial abnormality due to which a predisposition can be detected) can be considered as healthy. In other words, those for whom a predisposition for a disease treatable with folic acid can be detected and those for whom no such detection is possible, cannot be both considered as healthy.

In addition, it is clear to a skilled person in the art that the examples of the present application describe clinical pharmaceutical trials (to carry out said trials also clinical-ethical permission had to be procured). Accordingly, the terms "healthy" or "otherwise healthy" have to be discussed in this context, in other words, the question is what these terms mean for a skilled person if they are used in the description of a clinical trial.

According to a document of the National Institute of Health (USA), FAQs About Clinical Studies (copy enclosed), available over the internet at <http://clincialecenter.nih.gov/participate/faqaboutcs.shtml> on page 1, the definition of a healthy volunteer is given as follows:

3. What is a "healthy volunteer"? A volunteer subject with no known significant health problems who participates in research to test a new drug, device, or intervention is known as a "healthy volunteer" or "Clinical Research Volunteer." Accordingly, an individual who has medical problems to be treated with folic acid cannot be considered as healthy.

According to another document of the National Institute of Health, Patient

Information Publications, NIH Clinical Center, (copy enclosed), available over the internet at <http://clinicatecenter.nih.gov/participate/-pdf/partners.pdf>, the following information about healthy volunteers is given:

"We need to study healthy volunteers for several reasons: ... we need clinical research volunteers to help us define the limits of "normal"."

Accordingly, the group of healthy individuals forms the normal population, and the others can be defined as "higher than normal".

The clinical trials disclosed in the present specification are qualified as Phase I trials. US FDA defines the term "healthy" in the case of Phase I Clinical trials carried out with healthy volunteers according to Phase 1 Clinical Studies, FDA Handbook (copy enclosed) available over the internet at (<http://www.fda.gov/cder/handbook/phase1.htm>).

"Phase 1 includes the initial introduction of an investigational new drug into humans. These studies are closely monitored and may be conducted in patients, but are usually conducted in healthy volunteer subjects. These studies are designed to determine the metabolic and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness."

The next examples show what the term "healthy" means for a skilled person:
FAQs on GCP, Clinical Trial Management, (copy enclosed) and available over the internet at (<http://www.clininvent.com/clininvent/links/trial.html>)

"What is the US FDA definition of a healthy volunteer?

US FDA guidance on General Considerations for Clinical Evaluation of Drugs discussed this issue. The term "healthy" suggests that a volunteer should be free from abnormalities which could complicate interpretation of the experiment or which might increase the sensitivity of the volunteer to the toxic potential of the drug. A healthy volunteer is one who has no evidence of a clinical biochemical or investigational abnormality (based on physical examination, lab investigations (hematology, liver function, renal function, blood sugar, cholesterol, triglycerides)

and x-ray chest ECG.

US FDA guidance for the industry, "General Considerations for the clinical evaluation of drugs" suggests the following investigations for phase I volunteers..

- CBC including platelet, urinalysis, BUN or creatinine, liver function, FBS or 2 hour postprandial sugar, ECG.
- Any other investigations as per the profile of the drug under investigation. For example, for a study on anti-platelet drug, it would be desirable to check BT, CT, PT in the volunteers. The importance of these tests is two fold. Volunteers should be free from abnormalities which would complicate the interpretation of experiment or which might increase the sensitivity of the subject to the toxic potential of the drug."

According to ABPI (Association of the British Pharmaceutical Industry), Impact of the EU Directive on Ethical Review& Phase 1 Studies, Vol. 14, No. 1, (Feb. 2003) (copy enclosed) and available over the internet at

<http://archive.instituteofclinicalresearch.org/SMOSubCommittee/Phaseampact.pdf> healthy volunteers can be selected on the basis of full medical history and laboratory tests (page 2, paragraph 4).

According to the homepage of the University of Surrey (Guildford), (copy enclosed) and available over the internet at (<http://www.open.mis.surrey.ac.ukdmisweb/modules/7431.htm>) "Definition of a healthy volunteer (appropriate pre-study test, and considerations of the limits of 'normality' for laboratory data, cardiovascular data)."

Accordingly, the term "healthy" mentioned in the disclosure of the clinical trials in the present application means that at the beginning of the examinations (i.e. at the beginning of the hormone-vitamin treatment) the participants of the trials had no such biochemical abnormality which would have required chronic folic acid treatment (therapeutic or preventive). In other words, the meaning of the term "healthy" in the context of a clinical pharmaceutical trial is well-known for a skilled person: neither those who are afflicted with, nor those who are

predisposed to become afflicted with a disorder due to which they require a continuous (preventive) treatment fall into this category. Obviously, the exact interpretation of the examinations would have been impossible if individuals had been involved who had been given folic acid not only to avoid the side effect of the hormone but they had been in need of folic acid treatment anyway.

Also according to Section 3.2.1. of a document of The European Agency for the Evaluation of Medicinal Products (EMEA), Note for Guidance on the Investigation of Bioavailability and Bioequivalence, and available over the internet at (<http://www.emea.eu.int/pdfs/human/ewp/140198en.pdf>)

healthy volunteers are selected on the basis of medical history and relevant laboratory tests. Therefore, those who are afflicted with, or those who are predisposed to become afflicted with a disorder due to which they require a continuous (preventive) treatment do not fall into this group, as the existing illness or the predisposition can be detected on the basis of the medical history and the relevant laboratory tests.

According to the definition given on the homepage of the International Psoriasis Community, a 501(c)(3) Organization, and appearing on the internet at (<http://www.psoriasissupport.org/isp/default/pnint.asp?laug=1&cont=101>) the definition of a "healthy volunteer" is the same as given by the NIH (see above), which evidences the generic and accepted nature of the definition. This site also defines a "patient volunteer": "A volunteer subject with a known health problem, who participates in research to better understand, diagnose, treat, or cure a particular disease or condition."

On the basis of the definitions given for "healthy volunteer" and for "patient volunteer", the patients of KAFRISSEN et al and those of the present invention can be particularly well separated:

Healthy volunteer	Patient volunteer
no known health problem	known health problem
to test a new drug, device or intervention	to ... cure a particular disease or condition

Accordingly, for patient volunteers there is a disease to be treated (KAFRISSEN et al), for healthy volunteers there is no known health problem.

According to the information given on the home page of GlaxoSmithKline, (copy enclosed) available over the internet at

http://www.gsk.com/responsibility/cr_issues/ct_healthy_volunteers.htm

"volunteers normally do not have the disease or condition, i.e they are healthy volunteers."

This example shows that the term "healthy" is interpreted by the second largest pharmaceutical company of the world, and, thus, by the skilled person, as including not only the absence of disease, but also the absence of other condition, such as absence of predisposition to become afflicted with a disorder.

According to the official homepage of the US FDA (<http://www.fda.gov/>) the term "otherwise healthy" is present in 929 documents of the US FDA. From said documents one is selected and enclosed to evidence that the skilled person understands said term according to our interpretation detailed above.

The term "otherwise healthy patient" in indications other than contraception (e.g. in the case of hormone therapy), means that apart from the indication of the hormone therapy the patient is healthy (no other illnesses or predispositions). This definition is well known to those skilled in the art. Appellant points to page 2 of the US Food and Drug Administration, Equality in Clinical Trials Drugs and Gender, Judith Levine Willis, where it is disclosed that a patient infected with HIV is categorized as otherwise healthy.

In response to the questions raised by the Examiner on page 2 of the office action of 4 May 2006 concerning headache, it is submitted that headache is neither an illness nor a predisposition, but a symptom. A person having a headache may be healthy or may be ill. The question whether someone is healthy or not can be answered on the basis of results of examination and laboratory tests. Patients having normal results are healthy, and patients having abnormal results (e.g. higher than normal) are not healthy.

As for the argumentation of the Examiner in paragraph 1 of page 7 of the office action of 4 May 2006, the following is submitted: Comparing males over the age of 65 with younger males, in the first group more individuals are afflicted with prostate cancer or more individuals have risk factors due to which they are predisposed to become afflicted with prostate cancer than in the second group. It does, however, not mean that all male individuals over the age of 65 are afflicted with or become predisposed to become afflicted with said disorder. The question whether someone is healthy or not can be answered on the basis of results of physical examination and laboratory tests. In general, in each age group there is an illness which occurs more frequently than in other age groups; however, it would be a false conclusion drawn from this fact that there exists no healthy individual.

It is believed that on the basis of the references provided it is obvious that those who require a continuous (therapeutic or preventive) folic acid treatment as in KAFRISSEN et al, or those who have diabetes as in SPELLACY et al, or have cervical dysplasia as in BUTTERWORTH et al, cannot be considered healthy. The NIH and FDA references discussed hereinabove unambiguously state that there exist healthy patients and that the term "healthy" cannot be considered indefinite.

The patients of KAFRISSEN et al have to take folic acid in any case, as they are afflicted with or predisposed to become afflicted with a disorder treatable by folic acid. The patients treated according to the presently claimed invention have to take folic acid only if they also must take a gestagen hormone; otherwise they should not take folic acid. Whether a patient

falls within the scope of the method of treatment disclosed in KAFRISSEN et al, within the scope of the presently claimed method of treatment, or within the scope of a third group, can be unambiguously decided on the basis of medical examination and laboratory tests.

Namely, on the basis of said medical examinations and laboratory tests, the patients are grouped into the following groups:

- patients with normal results fall within the scope of the patients treated according to the present invention;
- patients with abnormal results fall within the scope of the KAFRISSEN et al method if the abnormal results show that the individual is afflicted with or predisposed to become afflicted with a disorder treated by folic acid administration; and
- patients with abnormal results, wherein the abnormality is not connected to disorders treated by folic acid belong to a third group.

Thus in the Appellant's view it is not only that his patients treated according to the present method do not fall within the scope of KAFRISSEN et al, but furthermore there is even a third group of patients. In any event there is no disclosure or suggestion in KAFRISSEN et al to administer a plasma homocysteine reducing agent to the particular otherwise healthy patient according to the presently claimed invention to prevent the elevation of the patient's plasma homocysteine levels resulting from administration of a gestagen hormone. Thus none of claims 9, 11, 20, 27, 32 through 36 and 39 should be rejected under 35 USC 102 as anticipated or under 35 USC 103 as obvious in view of this reference.

KAFRISSEN et al is not an effective prior art reference against any claim on appeal view of Appellant's submission of a Declaration Under 37 CFR 1.131.

Appellant turns to the Examiner's refusal to accept the Declaration Under 37 CFR 1.131 that Appellant filed together with his amendment of 9 February 2006. See the Evidence Appendix. Appellant filed the declaration to show that he conceived of the invention well before

the effective date of KAFRISSEN et al as a reference under 35 USC 102e and then diligently reduced his invention to practice. By submitting the evidence of conception of his invention prior to the effective date of KAFRISSEN et al as a reference under 35 USC 102e/103 and of diligent reduction of his invention to practice, Appellant has removed the Examiner's basis for maintaining a rejection of the claims under this section of the statute. Therefore 35 USC 102e/103 should no longer be a basis to apply the KAFRISSEN et al reference against any claim in this application.

Appellant understands that the Examiner has maintained his rejection of claims 9, 11, 20, 27, 32 through 36 and 39 under 35 USC 102e in view of KAFRISSEN et al relying on MPEP 706.02(b)(D) which essentially states that a Declaration Under 37 CFR 1.131 may not be used to antedate an issued US Patent or published US Patent Application where the claims are directed to the same invention or to obvious variants thereof. The Examiner takes the position that the claims of KAFRISSEN et al either overlap with the Appellant's claims or are obvious variants of the Appellant's claims. Appellant disagrees.

Appellant believes that no claim now in the application should be rejected under 35 USC 102e in view of KAFRISSEN et al since the patient treated according to all of the present claims is a different patient from the patient treated according to KAFRISSEN et al. All of the patients treated according to KAFRISSEN et al are patients who are in need of folic acid treatment and so they are not healthy patients. There is no indication even in claims 9 and 11 now presented that the patients treated according to the present invention are not healthy patients. Appellant especially believes that claims 20, 27, 32 through 36 and 39, all directed to a method of reducing a risk in an otherwise healthy patient of thromboembolism induced by administration of a gestagen hormone by administering to the patient a therapeutically effective amount of a plasma homocysteine reducing agent, should not be rejected as anticipated by KAFRISSEN et al, and in fact believes that KAFRISSEN et al is not even an effective reference against these claims.

As explained hereinabove, the patient treated according to KAFRISSEN et al is not an "otherwise healthy patient" but is a patient whose members are afflicted with, or predisposed to become afflicted with, a disorder at a higher than normal incidence. Appellant has explained hereinabove why there is no overlap between the patient defined in KAFRISSEN et al and the patient treated according to the presently claimed invention. Appellant has also explained why it would not be obvious from KAFRISSEN et al to treat the otherwise healthy patient according to the present invention to prevent a risk of thromboembolism according to claims 20, 27, 32 through 36 and 39. Therefore with regard to claims 20, 27, 32 through 36 and 39 there is no reason why the Declaration Under 37 CFR 1.131 should not be accepted and KAFRISSEN et al removed as an effective reference.

Claims 20, 28, 29, 31, 32, 33, 35, 37 and 39 should not be rejected under 35 USC 102 as anticipated or under 35 USC 103 as obvious in view of SPELLACY et al.

SPELLACY et al reference provides no basis to reject claims 20, 28, 29, 31, 32, 33, 35, 37 and 39 now presented as either anticipated under 35 USC 102 or as obvious under 35 USC 103. The patients disclosed in SPELLACY et al are not the same healthy patients that are treated according to the present invention and there is no suggestion in SPELLACY et al to treat the otherwise healthy patients according to the presently claimed method to prevent elevated levels of plasma homocysteine resulting from the gestagen hormone treatment.

The patients of SPELLACY et al became diabetic in the course of the gestagen treatment. On the contrary, as it has been already detailed, the patients of the present invention are healthy - apart from the indication of the hormone therapy - at the beginning of the treatment and remain healthy in the course of the hormone treatment. Diabetes is not an indication of hormone treatment, therefore, the patients of SPELLACY et al and those of the invention are separate.

SPELLACY et al suggests that diabetic patients take Vitamin B6 together with

their contraceptive. According to the teaching of the present invention, non-diabetics also have to take Vitamin B6 together with the contraceptive.

SPELLACY et al does not teach that healthy patients also have to take Vitamin B6 together with the contraceptive, as in the tests disclosed therein the patients who were either already ill with elevated blood glucose levels, or became ill (patient 10) had started hormone therapy already 3-6 months before they started to take also vitamins (page 266, paragraph 3, page 267 paragraph 1, and Table 1 on page 268). In other words, those who were treated with hormone+vitamin were ill patients.

While most of the patients who take contraceptives do not become diabetic as a result of the hormone treatment, in the case of SPELLACY et al, all of the patients became diabetic. This fact suggests that the patients of SPELLACY et al were in fact predisposed to become diabetic even before the start of the treatment solely with the gestagen hormone (without Vitamin B6).

Thus there is no basis to reject any of the claims on appeal as anticipated under 35 USC 102 or as obvious under 35 USC 103 in view of SPELLACY et al.

Claims 20, 27, 32, 33, 34, 35 and 39 should not be rejected under 35 USC 102 as anticipated or obvious under 35 USC 103, in view of BUTTERWORTH et al.

The BUTTERWORTH et al reference provides no basis to reject any of claims 20, 27, 32, 33, 34, 35 and 39 as anticipated under 35 USC 102 or as obvious under 35 USC 103. Reference is made to page 74, Materials and Methods, paragraph 1 of BUTTERWORTH et al according to which "Candidates for study were selected from a population of young women referred to a Public Health Colposcopy Clinic for evaluation of an abnormal cervical smear".

Accordingly, the patients of BUTTERWORTH et al had cervical dysplasia already at the beginning of the folic acid treatment, therefore, they were not healthy. In other words first they were treated solely with a gestagen hormone, due to which they have become

afflicted with cervical dysplasia, and afterwards they started to take also folic acid.

According to the last sentence of the abstract, "...such a derangement [in folate metabolism] is an integral component of the dysplastic process that may be arrested (not prevented!) or in some cases reversed by oral folic acid supplementation." Accordingly, in contrast to the opinion of the Examiner, BUTTERWORTH et al does not suggest that healthy patients undertake folic acid treatment for prevention of any disease. This is further confirmed in the Discussion part (page 81, column 2, penultimate line of paragraph 1): "The present study findings suggest that until the situation is further clarified an evaluation of nutritional status regarding folic acid would also be appropriate in patients with early cervical intraepithelial neoplasia."

The patients of BUTTERWORTH et al became afflicted with cervical dysplasia in the course of the hormone treatment. On the contrary, as it has been already detailed, the patients treated according to the presently claimed method are healthy - apart from the indication of the hormone therapy - at the beginning of the treatment and remain healthy in the course of the hormone treatment. Cervical dysplasia is not an indication of hormone treatment, therefore, the patients of BUTTERWORTH et al and those of the invention are separate, and so the reference is not anticipatory. Nor is there any suggestion in BUTTERWORTH et al to treat the otherwise healthy patients according to the present invention with both a gestagen hormone and folic acid. In the advisory action the Examiner himself acknowledged that "it is easy to understand what healthy patients might be". Appellant understands that in this respect the Examiner has changed his mind since the issuance of the final rejection and this revised opinion of the Examiner can be considered as further support of Appellant's argumentation included also in the appeal brief concerning the term "healthy".

In the advisory action the Examiner argues that he gives the broadest reasonable interpretation of the term "otherwise healthy", and, accordingly,

he considers the patients of Butterworth also as "otherwise healthy", because other than the oral contraceptive induced condition, they are healthy. In Appellant's view said interpretation is not broad, but incorrect. This view is supported by the argumentation (and supporting documents) included already in the appeal brief, namely, that in the context of the present patent description a skilled person understands that an otherwise healthy patient is healthy apart from the indication, for which the gestagen was prescribed. Therefore, a patient who became ill (e.g. with cervix dysplasia or diabetes) due to gestagen therapy (which is prescribed e.g. for any of the indications listed in claim 9 or 34) cannot fall into this group, as the gestagen has not been prescribed for cervix dysplasia or diabetes. Gestagens cannot be used for the treatment of cervix dysplasia or diabetes. Thus the patients treated according to BUTTERWORTH et al and treated according to the presently claimed intention are clearly not the same.

Thus no rejection of any claim on appeal should be maintained under either 35 USC 102 or 35 USC 103 in view of BUTTERWORTH et al.

Claims 9 through 12, 20, and 25 through 39 should be rejected under 35 USC 103(a) as unpatentable over US Patent 5,654,011 to JACKSON in view of FERMO et al

The combination of FERMO et al and JACKSON et al is in no way suggestive of the presently claimed method of treatment 9 through 12, 20, and 25 through 39. According to the Examiner, on the basis of the combination of FERMO et al and JACKSON et al the present invention is obvious. FERMO et al discloses only that there is a connection between elevated levels of plasma homocysteine and the development of thrombosis. There is first of all no disclosure or suggestion in either FERMO et al or JACKSON et al to administer a gestagen hormone together with a plasma homocysteine reducing agent. Nor is there any connection

disclosed or suggested in either reference of any correlation between elevated plasma homocysteine levels and administration of a gestagen hormone. Nor is there any indication that administration of a plasma homocysteine reducing agent to an otherwise healthy patient receiving gestagen hormone therapy would prevent thromboembolism in the patient.

Appellant does not agree with the Examiner's conclusion of the obviousness of the present claims in view of these two references in combination and as support for the unobviousness of the present invention, Appellant has made of record in the Response Under 37 CFR 1.116 abstracts of the following references: J. Chromatogr. 1986, Oct. 31; 382:247-52; Scand J Clin Lab Invest. 1992, Jun; 52(4):283-7; and Obstet Gynecol., 1999, Oct;94(4):485-91. A copy of the Scand J Clin Lab Invest. Article in its entirety was made of record earlier in the prosecution of this application as well. Said documents disclose that hormones do not cause elevated homocysteine levels. Therefore, a skilled person reading FERMO et al and JACKSON et al, either individually or in combination, would conclude that hormone treatment does not effect the homocysteine status of the patient. Thus, it is not suggested to take any homocysteine reducing agent in a hormone treatment.

JACKSON et al suggests the combination of vitamins and minerals for perimenopausal woman in order to prevent or reduce "life stage associated health risks". As taking gestagen hormones is not a life stage associated health risk, it is believed that this citation does not give any guidance to a skilled person to administer to a patient the present method of treatment of the present invention including both a gestagen hormone and a plasma homocysteine reducing agent.

In addition JACKSON et al suggests the administration of 400-440 μ g of folic acid, the invention suggests 500 μ g to 5 mg.

Appellant would like to draw the attention that, according to page 5, lines 19-26 of the specification, while the plasma homocysteine concentration is diminished by folic acid by 45-50% in hyperhomocysteinaemia of genetic origin (according to the state of the art), it is

diminished by 69% in hyperhomocysteinaemia induced by hormones (according to the invention, see also Example c) on page 8, line 13). In biological systems this difference in the effectiveness of the treatments is considered as significant. Therefore not only was the ability of the plasma homocysteine reducing agents to lower plasma homocysteine levels elevated as a result of taking gestagen hormones surprising in its own right, but furthermore the extent of the effectiveness of reducing plasma homocysteine levels was surprisingly high irrespective of the underlying cause of the plasma homocysteine elevation, including the known underlying genetic cause.

Accordingly no rejection of any of these claims should be maintained under 35 USC 103.

In paragraph 3 of page 3 of the Advisory Action of 3 October 2006, the Examiner argues that "a skilled person would have been motivated to employ the homocysteine (HCY) reducing agents to treat the elevated HCY and thereby reduce the risk of thromboembolism, regardless of the cause of the elevated HCY level." Appellant disagrees.

For a person skilled in the art, it is unambiguous that folic acid (and the other HCY reducing agents) are ineffective in most of the thromboembolisms: e.g. thromboembolisms caused by tumor, trauma, immobilization, inflammation, radiation or hypotyreosis. Before the priority date of the present application, namely, 1 February 1999, HCY reducing therapy had not been shown unambiguously effective in any of the thromboembolisms caused by hormones (including other hormones not only gestagens).

Furthermore, as it has been evidenced by the J. Chromatogr. 1986, Oct. 31; 382:247-52; Scand J Clin Lab Invest. 1992, Jun; 52(4):283-7; and Obstet Gynecol., 1999, Oct;94(4):485-91 references, discussed hereinabove, as of 1 February 1999 it had been believed by those skilled in the art that gestagens or oral contraceptives do not elevate the plasma HCY level. Therefore, in the absence of specific experimental results, the skilled person in the art would not have been motivated to use HCY reducing agents in order to reduce the risk of thromboembolism caused by gestagens. In other words the prior art teaches away from the method of treatment of the invention.

Appellant has found that contrary to the teaching of the prior art, the method of treatment according to the invention is effective in reducing the risk of thromboembolism caused by administration of gestagens.

As HCY reducing agents are effective in a very limited fraction of the thromboembolisms, only, the class of HCY reducing agents is a reverse case than that of morphine referred to by the Examiner in the Advisory Action, which has been found to reduce all kinds of pain (with diverse efficiency). Therefore, while, on the basis of the above, the skilled person would not have been motivated to use HCY reducing agents in order to reduce the risk of thromboembolism caused specifically by administration of gestagens, morphine would be expected to be effective against any kind of pain.

Appellant emphasizes that the Examiner did not cite any document which discloses the method of treatment of the invention. If the method of treatment of the invention had been obvious for a skilled person for long time (as it is stated in the Advisory Action), a document disclosing said method of treatment could have been easily cited. Nowhere in the prior art is there either a disclosure of or a suggestion of continued HCY-lowering therapy used explicitly because of HCY-plasma level elevation resulting from gestagen treatment.

For the same reason the argumentation of the Examiner that in the application old and well-known agents are used for known indication (HCY reducing agents for reducing the risk of thromboembolism), is not correct, as HCY reducing agents are not used for reducing the risk of thromboembolism in general, but only in the case of certain thromboembolisms,

The Examiner's reference to the existence of a monosynapticus reflex on page 4 of the Advisory Action (i.e. if a skilled person sees high HCY (HHCY) then he prescribes HCY reducing agent) is not correct either. According to the above argumentation, it is allowed to order HCY in a certain scope of indications, only. This is, for example, malpractice to order a HCY reducing agent to a patient suffering from hypotyreosis, necessarily having also HHCY, instead of ordering thyroxin.

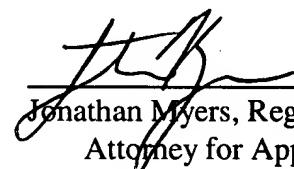
It is unambiguous from the J. Chromatogr. 1986, Oct. 31; 382:247-52 that HCY reducing agents are ineffective in most of the thromboembolisms and so it is believed that no claim now presented is obvious under 35 USC 103 in view of any of the cited prior art.

Thus no claim on appeal should be rejected under either 35 USC 112, second paragraph; 35 USC 102 or 35 USC 103.

Appellant respectfully requests that the US Patent and Trademark Office Board of Appeals and Interferences reverse the Examiner's rejection of all claims. Appellant has not included any payment for submission of this appeal brief in view of the fact that Appellant already paid the fee for submission of an appeal brief earlier in the prosecution of this application. The fee was paid on 19 July 2005 with the submission of an appeal brief. Following submission of the appeal brief, the Examiner reopened prosecution, and the appeal process was held in abeyance. The Examiner indicated at that time that should the Appellant resume the appeal process, there would be no need to pay once again either the fee for filing a Notice of Appeal or the fee for filing the Appeal Brief.

Since Appellant paid the fee for two Notices of Appeal in the same application, Appellant has requested a refund of the fee paid with the Notice of Appeal filed 29 September 2006. Appellant includes a Document Request for Refund with supporting papers, which is filed concurrently with this Appeal Brief.

Respectfully submitted,
The Firm of Karl F. Ross P.C.



Jonathan Myers, Reg. No. 26,963
Attorney for Appellant

er
November 29, 2006
5676 Riverdale Avenue Box 900
Bronx, NY 10471-0900
Cust. No.: 535
Tel: (718) 884-6600
Fax: (718) 601-1099

Enclosures: 131 Declaration & 20 References (listed in evidence appendix)
Document request for refund

(viii) CLAIM APPENDIX

1 9. A method of treating a patient undergoing treatment with a gestagen hormone
2 composition for hormone replacement therapy, for inflammation, for an in vitro fertilization
3 program, for dermatological therapy or for cosmetological treatment to reduce a risk to the
4 patient of thromboembolism induced by taking the gestagen hormone, which comprises the step
5 of administering to the patient simultaneously, previously or subsequently to taking the gestagen
6 hormone composition a therapeutically effective amount of a plasma homocysteine reducing
7 agent.

1 10. The method of treatment defined in claim 9 wherein the plasma homocysteine
2 reducing agent is a compound selected from the group consisting of Vitamin B₁₂, betaine,
3 choline, and acetylcysteine.

1 11. The method of treatment defined in claim 9 wherein the plasma homocysteine
2 reducing agent is folic acid.

1 12. The method of treatment defined in claim 9 wherein the plasma homocysteine
2 reducing agent is Vitamin B₆.

1 20. A method of reducing a risk to an otherwise healthy patient of
2 thromboembolism induced by administration of a gestagen hormone to said patient comprising
3 the step of administering to the patient simultaneously, previously or subsequently to taking the
4 gestagen hormone composition a therapeutically effective amount of a plasma homocysteine
5 reducing agent.

1 25. A method of treating a patient taking a composition comprising a gestagen
2 hormone to reduce a risk of thromboembolism induced by taking the gestagen hormone, which
3 comprises the step of administering to the patient simultaneously, previously or subsequently to
4 taking the composition comprising the gestagen hormone, a therapeutically effective amount of
5 Vitamin B₁₂, betaine, choline or acetyl cysteine.

1 26. The method of reducing a risk of thromboembolism defined in claim 20
2 wherein the plasma homocysteine reducing agent is a compound selected from the group
3 consisting of Vitamin B₁₂, betaine, choline, and acetylcysteine.

1 27. The method of reducing a risk of thromboembolism defined in claim 20
2 wherein the plasma homocysteine reducing agent is folic acid.

1 28. The method of reducing a risk of thromboembolism defined in claim 20
2 wherein the plasma homocysteine reducing agent is Vitamin B₆.

1 29. A method of treating a patient undergoing treatment with a gestagen hormone
2 composition to reduce a risk to the patient of thromboembolism induced by taking the gestagen
3 hormone composition, comprising the step of administering to the patient simultaneously,
4 previously or subsequently to taking the gestagen hormone composition, a therapeutically
5 effective amount of a plasma homocysteine reducing agent other than folic acid.

1 30. The method of treatment defined in claim 29 wherein the plasma
2 homocysteine reducing agent is a compound selected from the group consisting of Vitamin B₁₂,
3 betaine, choline, and acetylcysteine.

1 31. The method of treatment defined in claim 29 wherein the plasma
2 homocysteine reducing agent is Vitamin B₆.

1 32. A method of reducing a risk of thromboembolism upon administration of a
2 gestagen hormone to an otherwise healthy patient who may be threatened by an elevated plasma
3 homocysteine level as a result of the administration of the gestagen hormone composition which
4 comprises the step of administering to the patient simultaneously, previously or subsequently to
5 the gestagen hormone, a therapeutically effective amount of a plasma homocysteine reducing
6 agent.

1 33. The method of reducing a risk of thromboembolism defined in claim 32
2 wherein the otherwise healthy patient is from a class of individuals whose plasma homocysteine
3 levels have been elevated by administering to the patient a gestagen hormone composition and
4 where the patient's plasma homocysteine level is reduced following administration of the plasma
5 homocysteine reducing agent.

1 34. The method of reducing a risk of thromboembolism defined in claim 33
2 wherein the patient has been administered the gestagen hormone composition for contraception,
3 for hormone replacement therapy, for inflammation, for an in vitro fertilization program, for
4 dermatological therapy or for cosmetological treatment.

1 35. The method of reducing a risk of thromboembolism defined in claim 33
2 wherein the plasma homocysteine reducing agent is selected from the group consisting of folic
3 acid, Vitamin B₆, Vitamin B₁₂, betaine, choline, and acetylcysteine.

1 36. The method of reducing a risk of thromboembolism defined in claim 9
2 wherein the plasma homocysteine reducing agent is folic acid administered in an amount of 0.5
3 to 5 mg/day.

1 37. The method of reducing a risk of thromboembolism defined in claim 29
2 wherein the plasma homocysteine reducing agent is Vitamin B₆ administered in an amount of 10
3 to 300 mg/day.

1 38. The method of reducing a risk of thromboembolism defined in claim 29
2 wherein the plasma homocysteine reducing agent is Vitamin B₁₂ administered in an amount of
3 300 μ g to 5 mg/day.

1 39. The method of reducing a risk of thromboembolism defined in claim 20
2 wherein the gestagen hormone is administered to the patient for contraception or for hormone
3 replacement therapy.

(ix) Evidence Appendix

Appellant enclose a copy of a Declaration Under 37 CFR 1.131. The declaration was submitted by Appellant together with his amendment of 9 February 2006 and was entered by the Examiner and considered by the Examiner on page 8 of the office action mailed 5 May 2006.

Appellant encloses copies of the following references submitted during the prosecution of this application to show that there was no known connection at the time that the present invention was made between the administration to a patient of a gestagen hormone and the elevation of blood plasma homocysteine levels as a result of taking the gestagen hormone. In fact it was believed at the time of the present invention that administration of a gestagen hormone to a patient had no effect on the plasma homocysteine level of the patient.

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2. U.S. Patent 6,562,790; 13 May 2003; Edmund Chein
3. J. Clin. Ultrasound, Feb. 2005, 33 - I. Kocijancic; Sonographic detection of physiologic pleural fluid in normal pregnant women
4. WO 00/51596; 8 Sept. 2000; Messadek
5. GlaxoSmithKline: Healthy volunteer studies (www.gsk.com)
6. Diet Pills Glossary of Weight Loss Terms (www.adipex-phentermine-diet-pills.com)
7. NIH Clinical Center: FAQ About Clinical Studies (www.clinicalcenter.nih.gov)
8. Phase 1 Clinical Studies (www.fda.gov)
9. ClinInvent: Clinical Trial Management (www.clininvent.com)
10. Impact of the EU Directive on Ethical Review & Phase 1 Studies; Vol. 14; No. 1; Feb. 2003

11. EMEA: Committee for Proprietary Medicinal Products; 26 July 2001 (www.eudra.org)
12. International Psoriasis Community (www.psoriasissupport.org)
13. Patient Information Publications NIH Clinical Center: Partners in Research
14. www.open.mis.surrey.ac.uk - Full Module Description
15. U.S. Food and Drug Administration: Equality in Clinical Trials Drugs and Gender
Judith Levine Willis FDA Consumer Special Report
16. J. Chromatogr. 1986 Oct. 31; 382:247-52: Analysis of homocysteine in human urine using high-performance liquid chromatography with electrochemical detection
(abstract only)
17. Scand J Clin Lab Invest. 1992, Jun; 52(4):283-7 (abstract and full text)
18. Obstet Gynecol. 1999, Oct;94(4):485-91 (abstract only)
19. Dev Med Child Neurol. 1990 Dec; 32(12)
20. Eur. J. Echocardiogr. 2005 July 18

It is noted that references 1 through 4, 19 and 20 were entered by the Examiner together with Appellant's Amendment filed 9 February 2006;

It is noted that references 5 through 16, 17 in abstract form, and 18 were entered by the Examiner together with the Appellant's Response After Final Rejection filed 4 August 2006.

IN THE U.S. PATENT AND TRADEMARK OFFICE

BEST AVAILABLE COPY

Inventor Gabor BOGYE MD, PhD
Patent App. 09/890,029
Filed 24 July 2001 Conf. No. 6045
For PHARMACEUTICAL COMBINATION OF
PROGESTERONE AND FOLIC ACID
Art Unit 1617 Examiner Hui, S
Hon. Commissioner of Patents
Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.131

I, Gabor BOGYE, a citizen of Hungary, residing at Frankel Leó u. 7, H-1027, Budapest, Hungary, declare as follows:

THAT I have been awarded the degrees of M.D. and Ph.D. from a university fully accredited in Hungary;

THAT I have several years of experience in the study of homocysteine metabolism and of the effects of gestagens on patients taking this class of drug;

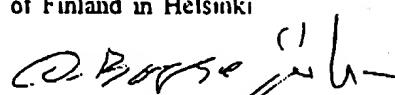
THAT my full curriculum vitae is attached hereto;

THAT I am the Applicant in US Patent Application Serial No. 09/890,029 filed 24 July 2001 and directed to PHARMACEUTICAL COMBINATION OF PROGESTERONE AND FOLIC ACID;

THAT I am aware that the Examiner in the US Patent Application has cited US Patent 6,190,693 to KAFRISSEN et al as prior art in the examination of my patent application as a basis for the anticipation of the claims therein and that the effective date of US Patent 6,190,693 as a reference under US law is 17 April 1998, the filing date of the Patent Provisional Application Serial No. 60/082,068;

THAT in order to establish that I conceived of the instant invention in my application on 3 June 1996 and that I diligently reduced the invention to practice on 1 February 1999, the filing date of my Hungarian Patent Application P9900213, I offer the following explanation supported by the attachments which accompany this declaration:

THAT during 1995, I received a scholarship through the Centre for International Mobility to conduct research at the National Public Health Institute of Finland in Helsinki where I conducted research in nutritional science;



THAT I enclose Attachment "A" to corroborate the scholarship (Attachment "A" is a letter from the Centre for International Mobility), extending the term of my scholarship;

THAT from the year of 1995 I partly personally conducted and supervised experiments on the plasma homocysteine status of otherwise healthy patients wherein some of them were taking a gestagen hormone. The corresponding data were compiled on 14 March 1996 and a data sheet was prepared a copy of which is attached as attachment "B". The data show that the administration to the healthy patient of a given dosage of a gestagen hormone (Tests G17, G21, and G39) resulted in elevated levels of plasma total homocysteine: 19.4, 20.3 and 27.8 μ mol/l, respectively. However, when healthy patients were administered the same dosage of gestagen hormone together with either Vitamin B₆ or folic acid together with the gestagen hormone (Tests G10 and G28, respectively), the plasma homocysteine concentrations were only 8.6 and 9.4 μ mol/l, respectively;

THAT based on the findings indicated above, I concluded that gestagens per se can elevate the plasma levels of homocysteine in otherwise healthy patients and that administering to that class of otherwise healthy patients, whose plasma homocysteine levels are elevated after taking gestagen hormones, Vitamin B₆ or folic acid results in a significantly lower level of plasma homocysteine;

THAT on 3 June 1996, on a copy of the above detailed data sheet I entered by hand the conclusions that I reached after analyzing the data (see Attachment "B"). I enclosed this paper to the research documentation;

THAT using the original data sheet on 4 June 1996 I prepared by hand a letter to the head of the laboratory where the plasma total homocysteine measurements were performed, a copy of said letter is attached as Attachment "B1" and contains the conclusions that I reached after analyzing the data;

THAT the remaining data in the data sheet have no bearing on my discovery that administration of gestagen per se may increase plasma homocysteine levels and that administration of the gestagen together with folic acid or vitamin B₆ to the same class of patients resulted in a drop in plasma homocysteine levels;

THAT I have attached to this declaration a disclosure of the present invention in Hungarian (Attachment "C") with a certified English translation thereof (Attachment "C1") that I prepared and had notarized after having discussed my invention and its patentability with patent experts in Hungary in summer and autumn of 1998 to corroborate my earlier date of conception and to establish my diligence in seeking its reduction to practice;

THAT I prepared my Hungarian Patent Application P 9900213 and filed same pursuant to the International Convention on 1 February 1999 thereby establishing a constructive reduction to practice of my invention at least as early as that date;

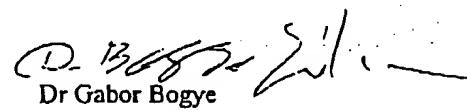
O. Bortz Sch

That attached to this declaration are Attachment "D", a certified copy of the Hungarian Patent Application P9900213, and Attachment "D1" a certified English translation thereof;

THAT I am aware of no information inconsistent with that presented above or which would lead one to a contrary conclusion; and

THAT I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under 18 USC 1001, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Budapest, 27.01.2006


Dr Gabor Bogye

Attachments A, B, B1, C, C1, D, D1.

2000 National Institute of Cardiology of Hungary "Laping Miklósné Award"
2000 Award of Hungarian Medical Chamber Association in Pest County
2003 National Institute of Rheumatology and Physiotherapy of Hungary
"Farkas Károly Award"

National Scientific Grants

1996 - 1999 OTKA (F-022296)
"Selenium status of five patients groups in Hungary"
1996 - 1999 ETT (224/96)
"Human selenium status in Hungary"
1999 OTKA (publishing grant)
1999 - 2001 OTKA (029832)
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Gyógyszereink, 1996, 2: 82-83.

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Palkonyai É, Larsen A, Geher P, Kolarz G, Bogye G, Temesvari P, Kaposi P, Szilagyi M, Ratko I.
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1992. ISTERN Third International Conference and NTES Fourth Nordic Conference on Trace Elements in Health and Disease, Stockholm

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1995. "Liver and Drugs" 1st International Symposium on Hepatology and Clinical Pharmacology (EASL, EACPT), Bratislava

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1995, Days of Hepatology, Liver and Drug Foundation, Stará Lesná, Slovakia

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1996. 16th Conference of Hungarian Association for Endocrinology and Metabolism, Debrecen.

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impact factor: 1.021

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(D2)



Attachment "A"

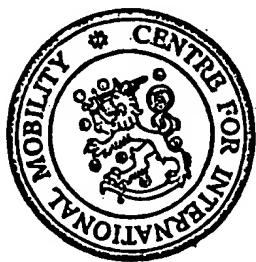
Helsinki, 13 July 1995

TO WHOM IT MAY CONCERN

This is to confirm that the Centre for International Mobility CIMO has granted Mr. Gábor Bogye 1 month's scholarship to enable him to continue his research at the National Health Institute of Finland in June 1996.

Ulla-Maija Anttila
Ulla-Maija Anttila

Exchanges Coordinator



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Attachment "B"

(D1)

KTL/RAVO/GA

14.03.96

PLASMA TOTAL HOMOCYSTEINE

Cys hom cg

Cys= cysteine umol/l 3 digits
 hom=homocysteine " 1 decimal
 cg=cysteinylglycine " 1 decimal

G1 1593183	192	6.4	23.5
G2 1593209	216	7.1	31.1
G3 1586824	187	6.8	22.3
G4 1587222	209	12.6	24.8
G5 1587233	224	10.4	21.1
G6 1586939	240	7.8	30.0
G7 1586818	179	5.4	26.9
G8 1587240	235	7.0	25.2
G9 1587045	270	7.7	27.6
G10 1586954	234	8.6	29.5
G11 1587279	282	10.3	26.4
G12 1586946	190	8.8	35.2
G13 1587198	251	8.0	29.5
G14 1587231	226	9.9	29.3
G15 1587223	236	8.4	31.9
G16 1587213	275	10.0	32.6
G17 1586943	264	19.4	24.0
G18 1587397	269	11.3	26.3
G19 1587244	181	11.0	24.7
G20 1587396	263	10.3	28.0
G21 1592622	210	20.3	24.2
G22 1586942	244	6.1	20.7
G23 1587227	239	7.8	22.1
G24 1587211	252	11.5	23.7
G25 1587242	236	7.1	29.1
G26 1586828	244	6.9	25.2
G27 1586957	230	10.4	22.1
G28 1587291	248	9.4	21.9
G29 1593277	203	5.8	24.1
G30 1593367	281	12.6	27.3
G31 1587260	234	8.7	35.0
G32 1593630	233	7.7	32.2
G33 1593149	240	6.5	18.1
G34 1587210	231	8.9	30.1
G35 1593359	247	8.6	31.8
G36 1593170	225	8.5	34.3
G37 1587245	258	14.0	35.0
G38 1593168	230	7.7	32.8
G39 1593132	268	27.8	33.5
G40 1593634	213	8.5	33.7
G41 1587927	208.028	7.5144	25.26
G42 1587925	292.986	10.1808	27.365
G43 1587921	237.406	6.6256	28.7122
G44 1593142	224.702	8.4032	25.8494
G45 1593200	269.96	10.4232	29.7226
G46 1593148	246.14	12.4432	30.0594

Point of: Healthy blood donor

G17 }
 G21 } each post. used gestagens
 G33 } /as contraceptive

G10 } also used gestagens but
 G28 } together with Vit B6
 and/or folic acid

Conclusions

① Gestagens can elevate plasma total HCY?
 ② Vit B6; folic acid prevent this gestagen effect?

Heimlich, 5.6. 1996.

Dr. Bayre jahn

Attachment "B1"
B1

KTL/RAVO/GA

14.03.96

Hungary, 04, 06, 1996

PLASMO. TOTAL HOMOCYSTEINE

Cys hom cg

Cys= cysteine $\mu\text{mol/l}$ 3 digits
 hom=homocysteine " 1 decimal
 cg= cysteinylglycine " 1 decimal

G1	1583183	192	6.4	23.5
G2	1583209	216	7.1	31.1
G3	1585824	187	6.8	22.3
G4	1587222	209	12.6	24.8
G5	1587233	224	10.4	21.1
G6	1586939	240	7.8	30.0
G7	1586818	179	5.4	26.9
G8	1587240	235	7.0	25.2
G9	1587045	270	7.7	27.6
G10	1586954	234	8.6	29.5
G11	1587279	282	10.3	26.4
G12	1586946	190	8.8	35.2
G13	1587198	251	8.0	29.5
G14	1587231	226	9.9	29.3
G15	1587223	236	8.4	31.9
G16	1587212	275	10.0	32.6
G17	1586943	264	19.4	24.0
G18	1587397	269	11.3	26.3
G19	1587244	181	11.0	24.7
G20	1587396	263	10.3	28.0
G21	1582622	210	20.3	24.2
G22	1586944	244	6.1	20.7
G23	1587227	239	7.8	22.1
G24	1587211	252	11.5	23.7
G25	1587247	236	7.1	29.1
G26	1586828	244	6.9	25.2
G27	1586957	230	10.4	22.1
G28	1587291	248	9.4	21.9
G29	1593277	203	5.8	24.1
G30	1593367	281	12.6	27.3
G31	1587260	234	8.7	35.0
G32	1593630	233	7.7	32.2
G33	1593149	240	6.5	18.1
G34	1587210	231	8.9	30.1
G35	1593359	247	8.6	31.8
G36	1593170	225	8.5	34.3
G37	1587245	258	14.0	35.0
G38	1593168	230	7.7	32.8
G39	1593132	268	27.8	33.5
G40	1593636	213	8.5	33.7
G41	1587927	208.028	7.5144	25.26
G42	1587925	292.986	10.1808	27.365
G43	1587921	237.406	6.6256	28.7122
G44	1593142	224.702	8.4032	25.8494
G45	1593200	269.96	10.4232	29.7226
G46	1593140	246.14	12.4432	30.0594

Dear Doctor,

On the basis of these results, I would like to recommend to continue the HCY study with patients taking estrogen/folate, and rosiglitazone, in vitro fertilization program.

Results

- Healthy patients (G17, G21, G35) taking estrogen hormone have elevated plasma total of HCY case.
- Healthy patients (G14, G26) taking estrogen hormone together with Vitamin B6 or folic acid have normal plasma total of HCY case.

Conclusion (n is small)

- Estrogen hormone can elevate plasma total of HCY case.
- Vit B6 or folic acid can prevent this effect of estrogen.

Dr. Bognar Csilla

P.S. Since elevated plasma HCY is a known risk factor for thromboembolism, my result may mean that Vit B6 or folic acid can prevent this side effect of estrogen (bleeding and rosiglitazone, hormone replacement therapy, in vitro fertilization, etc.)

Printed version of the handwritten text of attachment "B1"

Helsinki, 04, 06, 1996

Dear Georg,

On the basis of these results, I would like to recommend to continue the HCY study with patients taking gestagens /HRT, contraceptive, in vitro fertilization program/.

Results

- Healthy patients /G17, G21, G39/ taking gestagen hormone have elevated plasma total HCY conc.
- Healthy patients /G10, G28/ taking gestagen hormone together with Vitamin B6 or folic acid have normal plasma total HCY conc.

Conclusions /n is small!/

- Gestagen hormones can elevate plasma total HCY conc.
- Vit B6 or folic acid can prevent this effect of gestagens.

Dr Bogye Gabor

P.S.

Since elevated plasma total HCY is a known risk factor for thromb-embolism, my result may mean that Vit B6 or folic acid can prevent this side effect of gestagens /during contraception, hormone replacement therapy, in vitro fertilization, etc./.

Attachment C

DR. VÁRKONYI VERA KÖZJEGYZŐ
1027 Bp., Csalogány utca 21. I.3.
1536 Budapest, P1.280.
Tel/Fax: 213-9433

2.569/1998. ügyszám

Alulírott közjegyző tanúsítom, hogy DR. BOGYE GÁBOR (Szolnok, 1964.10.09., anyja neve: dr. Deutsch Stefánia) Budapest, I. ker. Berényi u. 8. földszint 1. szám alatti lakos - aki személyazonosságát a felmutatott AU-VII. 555493. számú személyi igazolványával igazolta - ezt az okiratot előttem saját kezűleg írta alá.

Budapest, 1998. Ezerkilencszázkilencvennyolcadik évi november hó 25.
Huszonötödik napján.



dr. Várkonyi Vera
közjegyző

Jelen szabadalmi összefoglalás közjegyzői hitelesítés céljából készült. A szabadalmi bejelentés kapcsán több szabadalmi ügyvivővel illetve szakértővel (a Szabadalmi Hivatalban Erkel András, Danubia Kft-ben Fehérvári Flóra és Molnár úr, továbbá Dr Machytka-Frank Daisy és édesapja Daisy otthonában) kellett már konzultálni. Ezben megbeszélések során részben ismertetni kellett a szabadalmat, ezért a szabadalmaztatni kívánt találmány jogi védelmét oly módon is biztosítani szeretné a szabadalom kizárolagos tulajdonosa, dr Bogyé Gábor, hogy közjegyző előtt hitelesíteti jelen okiratot.

"A hyperhomocystinaemia önálló rizikó faktora a vénás és artériás thrombembolia kialakulásának, mely az egyik legfontosabb mellékhatása a progeszteron és/vagy testosteron és/vagy az összes nemi hormon tartalmú gyógyszer készítményeknek. A fenti hormonok okozta szív és érrendszeri szövödmények a plazma homocystein tartalmának csökkentésével mérsékelhető (pl. folsavval, B6, B12-vitaminnal, betainnal, statinokkal és/vagy cholinnal). Ezek alapján előállíthatóak olyan hormon-tartalmú gyógyszer kombinációk, melyek a plazma homocystein szintet csökkentő adalékanyagokat tartalmazva az eredeti hatást megőrizve szignifikánsan kevesebb szív és érrendszeri mellékhatással bírnak. Ez egyszeri növeli ezen készítmények biztonságosságát, míg mások felhasználók köréi bővíti, hiszen számos olyan állapot, körkép létezik, melyek - a fenti szövödményekre tekintettel - eddig kizárták ezen hormon készítmények alkalmazását. Ezen hormon készítmények közé tartoznak például a fogamzásgátlók, a női és férfi "hormon replacement therapy" készítmények vagy az in vitro fertilitatio során felhasznált hormon tartalmú gyógyszerek."

Budapest, 1998. november 17.


Dr Bogyé Gábor

1016 Budapest, Berényi u. 8.

Hitelesítés a túloldalon

ORSZÁGOS FORDÍTÓ ÉS FORDÍTÁSHITELESÍTŐ IRODA



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H-1062 BUDAPEST, BAJZA U. 52.

3

1027 Budapest, Csalogány utca 21., I.3.

1536 Budapest, Pf. 280

Phone/fax: 213-9433

File No.: 2.569/1998

I, the undersigned notary public hereby certify
that DR BOGYE GÁBOR (Szolnok, 9 October 1964,
mother's name: Dr DEUTSCH Stefánia), residing at
Budapest 1st District, Berényi u. 8., groud-floor
1, who certified his identity with his identity
card No. AU-VII. 555493 presented before me,
signed this document before me in his own hand. ---
Budapest, this 25th (twenty-fifth) day of November
1998 (one thousand nine hundred and ninety-eight).

Illegible signature

Dr. VÁRKONYI Vera

Notary Public

L.S.: Dr. VÁRKONYI Vera, Notary Public, Budapest
77503/2006

The Hungarian National Office for Translations and Attestations Co. Ltd. hereby officially certifies that this photocopy is in full conformity with the Hungarian document presented as original.

Budapest, 05. 01. 2006

for the Director General - illegible signature

77503/2006

The Hungarian National Office for Translations and Attestations Co. Ltd. hereby officially certifies that this translation is in full conformity with the original document or the passage(s) marked therein, attached hereto.

Budapest, 05. 01. 2006

for the Director General
for the Director General



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National Office for Translations and Attestations Company Limited
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Általánosított Belépési Szempontúra és Műszaki
Bureau National de Traductions et de Legalisations Société Anonyme

H-1062 BUDAPEST, BAJZA U. 52.

Translation from Hungarian

The present patent summary has been drawn up for the purpose of authentication by a notary public. - Several patent managers and experts had to be consulted in connection with the patent application LŐKEL András in the Patent Office, FEHÉRVÁRI Móra and Mr. MOLNÁR at Danubia Kft., furthermore, Dr MACHYTKA Frank Daisy and his father Daisy in their home). In the course of the above discussions the patent had to be set forth in part, and therefore Dr BOGYE Gábor, the exclusive owner of the patent would like to ensure the legal protection of the invention intended to be patented in a way that the present document is authenticated by a notary public.

"Hyperhomocysteinaemia" is the independent risk factor of the development of venous and arterial thrombembolism, which is one of the most important side effects of pharmaceutical preparations of progesterone and/or testosterone contents, and/or of all pharmaceutical preparations of sex-hormones. The cardiac and vascular complications caused by the above hormones can be mitigated by reducing the homocystein contents of the plasma (e.g. with folic acid, Vitamins B6 and B12, be-

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taine, statines and/or cholin). On the basis of the foregoing, pharmaceutical preparations of hormone contents can be produced that have significantly less cardiac and vascular side effects since they contain additives reducing the homocysteine level of the plasma preserving the original effect. This increases the security of such preparations, on the one hand, and expands the sphere of users, on the other hand, since several conditions and clinical patterns exist, which excluded the application of the above hormone preparations until now, taking account of the above complications. Included in such hormone preparations are for instance: contraceptives, the hormone replacement therapy preparations for men and women, and the medicines of hormone contents used in the course of in vitro fertilisation." -----

Budapest, 17 November 1998-----

Illegible signature-----

Dr BOGYE Gábor-----

1016 Budapest, Berényi u. 8.-----

"Legalization overleaf"-----

Dr VÁRKONYI Vera-----

Notary Public-----



L



77503 05 /20 OFFICER

Hitelesen bizonyítom, hogy

ez a fénymásolat
másolat

az előttem eredetiként felmutatott

Macpherson
nyelvű írásban mindenben megegyezik.

Budapest, 20. 01. 2006

Általos Posta
Az Országos Fordító és
Fordításhitelesítő Irodá Rt.
vezérigazgatója

Attachment "D"

PCT/HU00/00009

**PRIORITY
DOCUMENT**

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)



REC'D	29 FEB 2000
WIPO	PCT

MAGYAR KÖZTÁRSASÁG

ELSŐBBSÉGI TANÚSÍTVÁNY

Ügyszám: P9900213

EPO - DG 1

12. 09. 2001

(71)

A Magyar Szabadalmi Hivatal tanúsítja, hogy

dr. Bogye Gábor, Budapest,

Magyarországon

1999. 02. 01. napján 4019/99 iktatószám alatt,

Gyógykompozíció(k) a hormon tartalmú készítmények szövődményeinek mérseklésére

című találmányt jelentett be szabadalmazásra.

Az idefűzött másolat a bejelentéssel egyidejűleg benyújtott melléklettel mindenben megegyezik.

Budapest, 2000. év 02. hó 11. napján

a Szabadalmi Főosztály vezetője

The Hungarian Patent Office certifies in this priority certificate that the said applicant(s) filed a patent application at the specified date under the indicated title, application number and registration number. The attached photocopy is a true copy of specification filed with the application.

P38 002/13

Elr. füld.

Gyógykompozíció(k) a hormon tartalmú készítmények szövődményeinek mérséklésére

1999 FEB 01

Dr. Bogye Gábor, belgyógyász, klinikai farmakológus

1027 Budapest, Frankel Leó u. 7.

Bejelentés napja: 1999. február 01.

KÖZZÉTÉTEL PÉLDÁNY

A találmány tárnya szteroid hormon(okat) és plazma homocisztein szintet csökkentő vegyülete(ke): tartalmazó olyan gyógykompozíció(k), amely(ek) alkalmazása során mérséklődik a hormon tartalmú készítmény(ek) thromb-embóliás szövődményeinek rizikója.

Ismeretes, hogy egyes hormonok, így például a szteroid hormonok terápiás alkalmazásának legfontosabb szövődménye, hogy növelik a thromb-embóliás megbetegedések gyakoriságát. Ezen szövődmények bármely szteroid hormont tartalmazó gyógyszer alkalmazásakor felléphetnek és gyakran halálos kimenetelűek.

Ezen probléma kezelésére eddig két eljárás volt ismert:

- Azon betegcsoportok, akiknél a thromb-embóliás szövődmények valószínűsége nagy (kövérök, dohányzók, 35 éves kor felett, körözményben thromb-embóliás megbetegedés), a hormon terápiából kizárástól kerülnek.
- Az alkalmazott hormon dózisának és a thromb-embóliás szövődmények gyakoriságának pozitív korrelációja alapján a gyógyszer készítmények hormon tartalmának mérséklésével csökkentették a thromb-embóliás szövődmények gyakoriságát.

Ezen ismert eljárások számos hátrányt mutatnak. Az abszolút és relatív kontraindikációk alapján a hormon terápiából kizárt betegek olyan kezeléshez nem jutnak hozzá, amely egyébként számukra szükséges. Az adott készítmény hormon tartalmának csökkentésével nemcsak az előforduló mellékhatások száma, hanem az élémi kivánt hatás mértéke is csökken. Mindkét ismert eljárás további hátránya, hogy a mellékhatások előfordulásának rizikója ugyan csökkenthető, azonban még a potenciálisan halálos mellékhatások sem zárhatóak ki teljes mértékben. Mindezek alapján igény van olyan új

20-130050-1.1

készítményekre, amelyeknél a szteroid hormon(ok) eredeti hatásának megtartása mellett a thromb-embóliák rizikója kisebb, mint az eddig ismert készítményeknél.

Az elmúlt évek kutatásai alapján vált ismerté, hogy a plazma homocisztein tartalmának növekedése önálló rizikó faktora a vénás és arteriás trombózisoknak és embóliáknak. Hivatkozunk az ide vonatkozó legújabb összefoglaló tanulmányokra (Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998, 338:1042-50.; den Heijer M, Kostor T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996, 334:759-62.; Graham IM, Daly IE, Refsum H, et al. Plasma homocysteine as a risk factor for vascular disease. *JAMA* 1997, 277:1775-81.).

Az EP 0347864 A2 számú szabadalmi bejelentésben (Strydom, Andries Johannes Cornelius: Anti-atherogenic agents. EP 0 347 864 A2.) foglaltak szerint egyes plazma és/vagy érfal homocisztein tartalmat csökkentő gyógykompozíciók alkalmazásával mérsékelhető az atherosclerosis. Ezen szabadalmi bejelentésben foglaltakkal szemben a magunk találománya arra épül, hogy a megnövekedett plazma homocisztein tartalom önmagában is hajlamosít thromb-embóliák kialakulására, még azelőtt, hogy érelmeszesedéshez vezetne. Találományaunkban a plazma homocisztein tartalom csökkenésének célja nem az atherosclerosis megelőzése vagy mérséklése, hanem az atherosclerosisról függetlenül is kialakuló thromb-embóliák kivédése.

Egyes vegyületek (folsav, B6 vitamin, B12 vitamin, betain, cholin, acetil-cisztein, penicillamine, adenosin és analógjai) ismerten csökkentik a plazma homocisztein tartalmat (Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998, 338:1042-50.; Refsum H, Ueland PM. Clinical significance of pharmacological modulation of homocysteine metabolism. *TiPS* 1990, 11:411-6.). Ezek közül a B vitaminok (folsav, B6 és a B12 vitamin) esetében már igazoltnak tekinthető, hogy mérséklik a nem gyógyszer indukálta thromb-embóliás szövödmények gyakoriságát (Graham IM, Daly IE, Refsum H, et al. Plasma homocysteine as a risk factor for vascular disease. *JAMA* 1997, 277:1775-81.; Herbert V, Bigaouette J. Call for endorsement of a petition to the Food and Drug Administration to always add vitamin B12 to any folate fortification or supplement. *Am J Clin Nutr* 1997, 65:572-3.).

20-13456-11

Találmányunk alapja az a felismerés, miszerint a szteroid tartalmú gyógyszerek alkalmazásával összefüggésbe hozható thromb-embóliák kialakulásáért részben a szteroid hormonok plazma homocisztein szintet növelő hatása a felelős. Vizsgálataink szerint különösen hatásosan emelik a plazma homocisztein koncentrációt a progeszteron típusú szteroidokat tartalmazó készítmények. A találmány további alapja az a felismerés, hogy ha a szteroid hormon(oka)t és a plazma homocisztein szintet csökkentő vegyületeket együttesen alkalmazzuk, akkor a hormon hatással kapcsolatba hozható össz plazma homocisztein tartalom növekedés szignifikánsan mérsékelhető, ami ismerten csökkenti a thromb-embóliás szövődmények rizikóját.

Találmányunk alapján előállíthatóak olyan gyógykompozíció(k), amely(ek) mérsékli(k) a hormontartalmú gyógyszerek thromb-embóliás szövődményeinek előfordulását.

Ezen gyógykompozíciók hatásos mennyiségben szteroid hormon(oka)t, ez(ek) metabolikus prekurzora(i)t, analógia(i)t és/vagy derivátuma(i)t továbbá a plazma homocisztein szintet csökkentő vegyülete(ke)t (például hatásos mennyiségű folsavat, B6 vitamint, B12 vitamint, betainit, cholint, acetil-ciszteint, penicillamint, adenosint, ez(ek) metabolikus prekurzora(i)t, analógia(i)t és/vagy derivátuma(i)t) tartalmaznak.

A gyógykompozíciók előnyös megvalósítási módja, hogy hormon komponense(i) fogamzásátára, hormonpótló terápiára (hormon replacement therapy), gyulladás-csökkentésre, az in vitro fertilitáció elősegítésére, bőrgyógyászati terápiára és/vagy kozmetológiai kezelésre alkalmas(ak) és a gyógykompozíciók is rendelkeznek ezen hatásokkal.

Jelenleg ilyen gyógykompozíciók nincsenek forgalomban.

a. példa: a fogamzásátálo hormonokat szedő nők átlagos össz plazma homocisztein tartalma nagyobb, mint a hormont nem szedő azonos korú nőké. A thromb-embóliás megbetegedések ismerten gyakrabban fordulnak elő a fogamzásátálo terápiában részesültek körében, mint a hormont nem szedő kontroll csoportban (Carr BR, Ory H. Estrogen and progestin components of oral contraceptives: relationship to vascular disease. (Review) Contraception 1997, 55:267-72.; Vessey MP. Benefits and risks of combined oral contraceptives (Review). Methods of Information in Medicine 1993, 32:222-4.). Vizsgálatunkba középkorú,

D. Bessy, M.

egészségesnek tekinthető véradó nőket vontunk be, akiket a fogamzásgátló szedés alapján két csoportba soroltuk majd megmértük az éhomi plazma össz homocisztein szintjüket HPLC módszerrel. A tartósan hormon terápiában részesültek átlagos össz plazma homocisztein tartalma szignifikánsan ($p<0.05$), átlagosan 3,2 $\mu\text{mol/L}$ -vel nagyobb volt, mint a kontroll csoporté.

b./ példa: középkorú nők átlagos össz plazma homocisztein tartalma szignifikánsan megnő a fogamzásgátló szedést követően. Vizsgálatunkba egészséges, középkorú nőket vontunk be, akiknél HPLC módszerrel megmértük a fogamzásgátló szedést megelőzően majd a kezelés megkezdését követően az éhomi plazma össz homocisztein tartalmat. Azt találtuk, hogy a plazma össz homocisztein tartalom szignifikánsan ($p<0.02$), átlagosan 3,7 $\mu\text{mol/L}$ -vel megnőtt a fogamzásgátló kezelés alatt.

c./ példa: egészséges, középkorú nők körében végzett vizsgálatunk szerint a fogamzásgátló szedés mellett észlelhető éhomi átlagos össz plazma homocisztein szint HPLC módszerével mérve szignifikánsan ($p<0.05$) kisebb, ha a fogamzásgátló szedés mellett folsavat (3 mg/die) vagy B6 vitamint (20 mg/die) is szedtek a résztvevők.

d./ példa: középkorú nők azon csoportjában, akik a recidív kismedencei gyulladásra való hajlamukat figyelembe véve részesültek hormon kezelésben, az éhomi plazma össz homocisztein szintje HPLC módszerrel mérve szignifikánsan ($p<0.05$) nagyobb volt, mint a hormonkezelésben nem részesült kontroll csoporté.

e./ példa: egészséges nők azon csoportjában, akik bőrgyógyászati, kozmetológiai indikációt figyelembe véve (pl. a hajhullás és a bőr zsírosodásának csökkentésére céljából) kaptak tartósan hormon kezelést, az éhomi plazma össz homocisztein szint HPLC módszerrel mérve szignifikánsan ($p<0.05$) nagyobb volt, mint a hormonkezelésben nem részesült kontroll csoportban.

f/ példa: az in vitro fertilitációs programban résztvevőknél mértük az éhomi plazma össz homocisztein koncentráció változását HPLC módszerrel. Arra az eredményre jutottunk,

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hogy a szteroid kezelés mellett szignifikánsan növekszik a plazma össz homocisztein koncentráció. A 3-9 mg/die dózisban alkalmazott folsav kezelés mellett a plazma homocisztein változás sokkal mérsékeltebb volt, mint azon esetekben, amikor párhuzamos folsav adagolás nem történt. A plazma homocisztein változás pozitív korrelációt mutatott a hormon kezelésekben alkalmazott progeszteron dózisokkal, vagyis feltételezhető, hogy a progeszteron a szteroid hormonok csoportján belül különösen hatásos a plazma homocisztein növelésében és ezen keresztül fokozottan felelős a thromb-embóliás megbetegedések rizikójának növekedéséért.

g/ példa: postmenopausaban az osteoporosis prevenciójára alkalmazott un. hormonpótló terápiában (hormon replacement therapy) részesültek körében végzett vizsgálataink szerint a kezeltek átlagos éhomi plazma össz homocisztein koncentrációja HPLC módszerrel mérve átlagosan 2,9 μ mol/l-vel nagyobb volt, mint a hormonpótlásban nem részesült kontroll csoporté.

Ezen felismerések azért meglepőek, mert eddig nem volt ismert, hogy a szteroid hormonok milyen patomechanizmus révén fokozzák a thromb-embóliás hajlamot, és hogy a szteroid hormonoknak direkt módon hatásuk lenne a szervezett plazma homocisztein háztartásra.

Ezen gyógykompozíció(k) előnye a csak hormoni tartalmazó készítményekkel szemben az, hogy kisebb a thromb-embóliás szövődmények rizikója. Ezen gyógykompozíció(k) révén a szteroid hormonok alkalmazása biztonságosabbá válhat és olyan betegcsoportok részére is rendelhetők (dohányzók, kővérek, 35 éves kor feletti, pozitív anamnesztikus adat esetén, stb.), akik a hormont tartalmazó készítmények alkalmazásából eddig ki voltak zárva. További előny, hogy a szteroid hormon(oka)t és a plazma homocisztein koncentrációt csökkentő vegyület(ek) egy kiszerelési egységben (tableta, kapszula, ampulla, por, oldat, granulátum, szirup, stb.) történő elhelyezése révén találmányunk biztosítja, hogy a hormon hatóanyagot és a plazma homocisztein szintet csökkentő, a hormon legfontosabb mellékhatása ellen ható "antidotum"-ot kizárolag csak együtt lehessen alkalmazni.

0-130-701/1

Szabadalmi igénypontok

1. Hormon tartalmú gyógykompozíció(k), ahol a gyógykompozíció(k) plazma homocisztein szintet csökkentő komponens(eket) tartalmaznak.
2. Az 1. igénypont szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy a plazma homocisztein szintjét csökkentő komponensként hatásos mennyiségű folsavat, B6 vitamint, B12 vitamint, betaint, cholint, acetilciszteint, penicillamint, adenosint, ez(ek) metabolikus prekurzora(i)t, analógia(i)t és/vagy derivátuma(i)t tartalmaz(zák).
3. Az 1. vagy 2. igénypont szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy hormon komponensként hatásos mennyiségű szteroid hormon(oka)t, szteroid hormon metabolikus prekurzora(i)t, analógia(i)t és/vagy derivátuma(i)t tartalmaz(zák).
4. Az 1-3. igénypontok bármelyike szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy hormon komponensként hatásos mennyiségű progeszteron hormon(oka)t, metabolikus prekurzora(i)t, analógia(i)t és/vagy derivátuma(i)t tartalmaz(zák).
5. Az 1-4. igénypontok bármelyike szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy fogamzásgátlásra szolgáló hormon(oka)t tartalmaznak.
6. Az 1-4. igénypontok bármelyike szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy hormonpólásra szolgáló hormon(oka)t tartalmaznak.
7. Az 1-4. igénypontok bármelyike szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy gyulladás-csökkentésre szolgáló hormon(oka)t tartalmaznak.
8. Az 1-4. igénypontok bármelyike szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy a fertilitáció elősegítésére szolgáló hormon(oka)t tartalmaznak.
9. Az 1-4. igénypontok bármelyike szerinti gyógykészítmény(ek) azzal *jellemzve*, hogy bőrgyógyászati, kozmetológiai kezelésre szolgáló hormon(oka)t tartalmaznak.

0-134551/11

Archivat "D1"

PHARMACEUTICAL COMPOSITION(S) FOR REDUCING THE SIDE EFFECTS OF COMPOSITIONS CONTAINING HORMONES

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The subject of the present invention is pharmaceutical composition(s) comprising steroid hormone(s) and compound(s) lowering the level of homocysteine of the plasma. During the use of such compositions the risk of the thromboembolic side effects of the hormone composition(s) is reduced.

It is known that the most important side effect of the therapeutic use of some hormones, for example, steroid hormones, is the increased occurrence of thromboembolic diseases. These side effects may occur upon the administration of pharmaceutical composition comprising any steroid hormone and they are often lethal.

This problem has been solved so far by two methods:

- a) Patients, in case of which the probability of the occurrence of thromboembolic side effects is high (adipose, smokers, patients of the age above 35, patients whose anamnesis already showed thromboembolic disease), were excluded from the hormone therapy.
- b) On the basis of the positive correlation of the occurrence of the thromboembolic complications and the dose of the hormone used, by decreasing the hormone content of the pharmaceutical compositions the occurrence of the thromboembolic complications was reduced.

These known methods show numerous disadvantages. The patients excluded from the hormone therapy on the basis of the absolute and relative contraindications, cannot obtain an otherwise necessary treatment. By reducing the hormone content of the given pharmaceutical composition, not only the occurrence of the side effects is reduced, but the extent of the effect to be achieved, too. A further disadvantage of

both known methods is that although the risk of the occurrence of the side effects can be reduced, even the potentially lethal side effects cannot be entirely eliminated. On the basis of these there is a need for compositions, which have a lower risk of thromboembolic diseases than the known compositions, while maintaining the original activity of the steroid hormone(s).

On the basis of recent research it has become known, that the increase of the homocysteine content of the plasma is an independent risk factor of arterial and venal thrombosis and embolism. We refer to the latest studies summarising this problem: (Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998, 338:1042-50.; den Heijer M, Kostor T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996, 334:759-62.; Graham IM, Daly IE, Refsum H, et al. Plasma homocysteine as a risk factor for vascular disease. *JAMA* 1997, 277:1775-81.).

In EP 0347864 A2 (Strydom, Andries Johannes Cornelius: Anti-atherogenic agents. EP 0347864 A2) it is disclosed that atherosclerosis can be reduced by using some pharmaceutical compositions reducing the homocysteine content of the plasma and/or arterial wall. As opposed to the contents of said patent specification, we have found that the increased homocysteine content of the plasma itself induces susceptibility to thromboembolism before leading to atherosclerosis. According to our invention, the aim of reducing the homocysteine content of the plasma is not to prevent or reduce atherosclerosis but to achieve the prevention of thromboembolism occurring also independently of atherosclerosis.

It is known that some compounds (folic acid, vitamin B₆, vitamin B₁₂, betaine, choline, acetyl cysteine, penicillamine, adenosine and its analogs) reduce the homocysteine content of the plasma (Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998, 338:1042-50.; Refsum H, Ueland PM. Clinical significance of pharmacological modulation of homocysteine metabolism. *TiPS* 1990, 11:411-6.) From these in the case of vitamins B (folic acid, vitamins B₆ and B₁₂) it can be considered as proven that they reduce the occurrence of thromboembolic complications not induced by medicines (Graham IM, Daly IE, Refsum H, et al. Plasma homocysteine as a risk factor for vascular disease. *JAMA* 1997, 277:1775-

81.; Herbert V, Bigaouette J. Call for endorsement of a petition to the Food and Drug Administration to always add vitamin B12 to any folate fortification or supplement. Am J Clin Nutr 1997, 65:572-3.).

We have found that the occurrence of thromboembolic diseases which can be correlated with the administration of pharmaceutical compositions containing steroid hormone, is partly due to the plasma homocysteine level increasing activity of the steroid hormones. According to our examinations, compositions containing progesterone-type steroids increase the plasma homocysteine concentration particularly effectively. The further basis of the invention is the recognition that if steroid hormone(s) and plasma homocysteine level reducing compounds are both used, then the total increase in the plasma homocysteine content which can be correlated with the hormone effect can be significantly reduced, which reduces the risk of the thromboembolic side effects.

On the basis of our invention pharmaceutical composition(s) can be prepared which reduce(s) the risk of the thromboembolic side effects of hormone containing medicines.

These pharmaceutical composition(s) comprise an effective amount of steroid hormone(s), metabolic precursor(s), analogue(s) and/or derivative(s) thereof, and plasma homocysteine level reducing compound(s) (e.g. effective amount of folic acid, vitamin B₆, vitamin B₁₂, betaine, choline, acetyl cysteine, penicillamine, adenosine and metabolic precursors, analogues and/or derivatives thereof).

In a preferred embodiment of the pharmaceutical compositions the hormone component(s) is (are) suitable for contraception, hormone replacement therapy, antiinflammation, promoting in vitro fertilisation, dermatological therapy and/or cosmetological treatment and the compositions also show these effects.

No such compositions are available on the market at present.

Example a)

The average total plasma homocysteine content of women taking contraceptives is higher than that of women of similar age not taking contraceptives. The thromboembolic diseases are known to occur more frequently in the case of women taking part in contraceptive therapy than in the case of control group not taking hormone (Carr BR, Ory H. Estrogen and progestin components of oral contraceptives: relationship to vascular disease. (Review) Contraception 1997, 55:267-72; Vessey MP. Benefits and risks of combined oral contraceptives (Review). Methods of Information in Medicine 1993, 32:222-4). Healthy blood-donor women of middle age were involved in the test, who were divided into two groups on the basis of taking contraceptives, and then their fasting plasma total homocysteine level was measured by HPLC method. The average total plasma homocysteine content of the women obtaining durable hormone therapy was significantly ($p<0.05$) higher, on average by $3.2 \mu\text{mol/L}$, than that of the control group.

Example b)

The average total plasma homocysteine content of women of middle age taking contraceptives significantly increases after taking contraceptives. Healthy women of middle age were involved in the test. The fasting total plasma homocysteine content was measured by HPLC method before starting the administration of contraceptive and after starting the treatment. We have found that during contraceptive treatment the total homocysteine content of the plasma significantly ($p<0.02$) increased, on average by $3.7 \mu\text{mol/L}$.

Example c)

According to our examinations with healthy women of middle age, the fasting average total plasma homocysteine level observable during taking contraceptives, determined by HPLC, is significantly ($p<0.05$) lower for patients who took not only the contraceptive, but also folic acid (3 mg/die) or vitamin B₆ (20 mg/die).

Example d)

According to our examinations with women of middle age obtaining hormone therapy due to their susceptibility to inflammation of small pelvis (*pelvis minor*), the fasting

total plasma homocysteine level measured by HPLC was significantly ($p<0.05$) higher, than that of the control group not obtaining hormone therapy.

Example e)

According to our examinations with healthy women obtaining durable hormone therapy for dermatological, cosmetological indications (e.g. to reduce alopecia and pinguefaction of the skin), the fasting plasma total homocysteine level measured by HPLC was significantly ($p<0.05$) higher, than that of the control group not obtaining hormone therapy.

Example f)

In the case of women taking part in *in vitro* fertilisation program, the change of fasting total plasma homocysteine concentration was measured by HPLC method. The result obtained is that by steroid treatment the total homocysteine concentration of the plasma significantly increases. By treatment with folic acid in the dose of 3-9 mg/die the change in the plasma homocysteine was much more moderate, than in those cases when parallel folic acid administration did not happen. The change of the plasma homocysteine showed positive correlation with the progesterone doses used in the hormone therapy, therefore, it can be assumed that progesterone, within the group of steroid hormones, particularly effective in increasing the plasma homocysteine, and, thus, it has great responsibility for the increase of the risk of thromboembolic diseases.

Example g)

According to our examinations with patients obtaining hormone replacement therapy for the prevention of osteoporosis in postmenopausa, the average fasting total plasma homocysteine concentration of the patients measured by HPLC was higher by 2.9 $\mu\text{mol/l}$ on average, than that of the control group not obtaining hormone replacement therapy.

These findings are surprising, as so far it has been not known by which patomechanism the steroid hormones increase the susceptibility to thromboembolism, and, that the steroid hormones have direct effect on the homocysteine household of the plasma.

The advantage of these pharmaceutical composition(s) over the compositions containing hormone only is the smaller risk of thromboembolic side effects. The use of steroid hormones can become safer by these pharmaceutical composition(s) and they can be prescribed for patient groups (smokers, patients above the age of 35, in case of positive anamnestic data, etc.), who were excluded from the use of compositions containing hormone. A further advantage is that by placing both the hormone(s) and the compound(s) reducing the plasma homocysteine concentration in one dosage unit (tablet, capsule, ampoule, powder, solution, granules, syrup, etc.), the invention ensures that the hormone active ingredient and the „antidote” acting against the most important side effect of the hormone, i.e. the plasma homocysteine reducing agent, can be used together/simultaneously, only.

Claims:

1. Pharmaceutical composition(s) containing hormone(s), comprising plasma homocysteine content reducing component(s).
2. Pharmaceutical composition(s) as claimed in claim 1, comprising as plasma homocysteine content reducing component folic acid, vitamin B₆, vitamin B₁₂, betaine, choline, acetyl cysteine, penicillamine, adenosine, metabolic precursors, analogues and/or derivatives thereof, in an effective amount.
3. Pharmaceutical composition(s) as claimed in claim 1 or 2, comprising as hormone component steroid hormone(s), metabolic precursor(s), analogue(s) and/or derivative(s) thereof, in an effective amount.
4. Pharmaceutical composition(s) as claimed in any of claims 1 to 3, comprising as hormone component progesterone hormone(s), metabolic precursor(s), analogue(s) and/or derivative(s) thereof, in an effective amount.
5. Pharmaceutical composition(s) as claimed in any of claims 1 to 4 comprising hormone(s) for contraception.
6. Pharmaceutical composition(s) as claimed in any of claims 1 to 4 comprising hormone(s) for hormone replacement.
7. Pharmaceutical composition(s) as claimed in any of claims 1 to 4 comprising hormone(s) for reduction of inflammation.
8. Pharmaceutical composition(s) as claimed in any of claims 1 to 4 comprising hormone(s) for promoting fertilisation.
9. Pharmaceutical composition(s) as claimed in any of claims 1 to 4 comprising hormone(s) for dermatological, cosmetological treatment.

CERTIFICATION OF TRANSLATION

I, Dr. Daisy Machytka-Frank, of Keleti Károly u. 13/b, H-1024 Budapest, Hungary, patent attorney with the firm of Gödölle, Kékes, Mészáros & Szabó Patent and Trademark Attorneys, Budapest, Hungary, hereby declare

- that I know well both the Hungarian and the English languages;
- that I have translated the attached Priority Document of Hungarian Patent Application No. P9900213 filed on 1 February 1999; and
- that to the best of my knowledge and belief the attached English translation is a true and correct translation of the document referred to above.

Budapest, 3 January 2006

Dr. Daisy Machytka-Frank



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(54) Title: ANTITHROMBOTIC USE OF GLYCINE BETAINE

(54) Titre: LA GLYCINE BETAINE POUR SON USAGE ANTITHROMBOTIQUE

(57) Abstract

The invention concerns the use of glycine betaine to eliminate physiopathological vascular diseases. The invention concerns the curative and preventive activity of glycine betaine in the pathogenesis of thromboembolic and hemostatic diseases of arterial or venous origin. Glycine betaine has a preventing activity by inhibiting the formation of thrombi and a curative activity inhibiting the proliferation of thrombi by eliminating them. The invention is characterised in that glycine betaine does not present any risk of haemorrhage or allergy contrarily to molecules and treatments currently used. The invention also concerns the use of glycine betaine as anticoagulant for blood preservation.

(57) Abrégé

L'invention consiste en l'utilisation de la glycine bétaine pour éliminer les atteintes physiopathologiques vasculaires. L'invention se rapporte à l'activité curative et préventive de la glycine bétaine dans la pathogénie des maladies thrombo-emboliques et hémostasiques d'origine artérielle ou veineuse. La glycine bétaine a une activité préventive en empêchant la formation des thrombi et une activité curative empêchant la prolifération des thrombi en les détruisant. L'intérêt de l'invention réside dans le fait que l'utilisation de la glycine bétaine ne présente aucun risque hémorragique ou allergique par opposition aux molécules et traitements actuellement utilisés. L'invention consiste également en l'utilisation de la glycine bétaine comme anti-coagulant pour la conservation du sang.

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LA GLYCINE BÉTAÏNE POUR SON USAGE ANTITHROMBOTIQUE.

5

10 L'invention consiste en l'utilisation de la glycine bétaine pour éliminer les atteintes physiopathologiques vasculaires. L'invention se rapporte à l'activité curative et préventive de la glycine bétaine dans la pathogénie des maladies thrombo-emboliques et hémostasiques d'origine artérielles ou veineuses.

15 La glycine bétaine a une activité préventive en empêchant la formation des thrombi et une activité curative empêchant la prolifération des thrombi en les détruisant. L'intérêt de l'invention réside dans le fait que l'utilisation de la glycine bétaine ne présente aucun risque hémorragique ou allergique par opposition aux molécules et traitements actuellement utilisés.

20

ETAT DE LA TECHNIQUE

25 Les thromboses vasculaires sont une réponse de l'organisme face à l'agression de la paroi du vaisseau et de son contenu cellulaire et plasmatique. La thrombose est l'activation localisée de la coagulation avec constitution d'un thrombus.

30 L'intérêt suscité par cette pathologie ces dernières années a permis d'incriminer plusieurs facteurs :

- Le vaisseau, la paroi vasculaire et les cellules endothéliales
- Le rôle des éléments figurés du sang
- Les systèmes de coagulation, de fibrinolyse, et leurs inhibiteurs.

35 Il existe plusieurs types de thromboses qui peuvent survenir au niveau des artères, des veines, de la microcirculation des organes, des cavités du cœur et des surfaces artificielles en contact avec le sang. Les thromboses vasculaires sont une réponse de l'organisme à l'agression de la paroi du vaisseau et de son contenu cellulaire et plasmatique. La thrombose est une masse organisée d'éléments sanguins (plaquettes, globules rouges et globules blancs), de fibrine et d'autres protéines plasmatiques, qui est déposée à la surface ou qui obstrue la lumière du système vasculaire. Les mécanismes de la thrombose ressemblent à ceux de l'hémostase, mais 40 sont pathologiques par leur localisation intravasculaire anormale.

45 Les thromboses et les embolies sont la cause principale des complications cliniques des maladies cardio-vasculaires et de l'athérosclérose.

D'après Virchow, au moins trois types de facteurs thrombogènes déterminent la localisation, l'extension et la régression d'une thrombose :

- Les facteurs hémodynamiques et rhéologiques;
- La lésion endothéliale;

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~~l'activation des constituants du sang, en particulier des plaquettes et de la coagulation qui aboutit à la formation de thrombine.~~

5 La maladie thrombo-embolique, d'origine artérielle ou veineuse reste une des causes principales de décès dans les pays développés.

10 La thrombose artérielle est souvent due à une rupture de la plaque d'athérosclérose alors que la thrombose veineuse résulte du déficit d'un inhibiteur de la coagulation (AT III) ou d'un déficit d'un activateur de la fibrinolyse (protéine S et/ou protéine C) ou plus fréquemment d'une stase. En effet, si tous les deux résultent d'une interaction entre le sang et la paroi 15 vasculaire, la formation d'une thrombose veineuse et/ou par une anomalie de l'hémostase. La thrombose artérielle est le plus souvent secondaire à une anomalie pariétale et implique principalement les plaquettes sanguines. Elle contribue à une large variété de tableaux cliniques selon les lits artériels intéressés par l'interruption de la vascularisation. La thrombose peut atteindre principalement les artères cardiaques (coronaires), les artères des membres inférieurs, 20 cérébrales ou digestives. Ainsi, la maladie artérielle favorise la formation du thrombus lui-même responsable de la majorité des occlusions vasculaires terminales. De plus la participation du désordre de l'hémostase et du thrombus formé à d'autres lésions vasculaires est manifeste : aggravation des lésions de la paroi, ischémie et troubles de la microcirculation.

25 On peut distinguer trois stratégies thérapeutiques dans la prévention des accidents liés aux thromboses.

25 Les anticoagulants. Ils constituent l'élément majeur de la prise en charge d'un patient présentant une affection thrombo-embolique. L'héparine et ses dérivés sont couramment utilisés. Cependant, l'utilisation des héparines peut engendrer deux complications majeures, l'hémorragie ou la thrombopénie.

30 Les anti-vitamines K (AVK). Prescrites pour des traitements au long cours, elles ne peuvent être utilisées dans l'urgence et ne peuvent être prescrites simultanément avec d'autres anti-agrégants dont elles potentialisent l'effet hémorragique.

35 Les anti-agrégants plaquettaires. Prescrits pour prévenir la thrombose artérielle liée à l'athérosclérose. Actuellement les principaux inhibiteurs du fonctionnement plaquettaire prescrits sont : l'aspirine, la ticlopidine, le dipyridamole, et certains anti-inflammatoires non stéroïdiens comme le flurbiprofène et la prostacycline. Ces traitements possèdent une réelle efficacité toute en présentant des effets indésirables sur les patients à terrains allergiques ou hémorragiques.

40 Tous ces traitements malgré leur efficacité nécessitent des précautions particulières dans leurs utilisations, telles que l'administration d'antidotes, les problèmes de surdosages et les effets secondaires non désirables. Ces traitements impliquent un suivi nécessaire des patients, dû notamment aux problèmes hémorragiques qui peuvent survenir pendant ou après la médication ainsi qu'aux éventuelles incompatibilités avec d'autres médicaments. Il était donc intéressant de trouver une molécule à haut potentiel antithrombotique sans effets indésirables. De manière tout à fait surprenante la glycine bétaine est apparue comme possédant un haut potentiel thérapeutique dans le traitement des thromboses.

45 La glycine bétaine ou bétaine de formule $(CH_3)_3N-CH_2-COOH$ est une molécule connue pour ses propriétés osmoprotectrices ainsi que pour ses utilisations cosmétiques et pharmaceutiques. Diverses applications pharmaceutiques de la bétaine sont connues et en particulier l'utilisation de la bétaine pour le traitement de l'homocystéinurie cause de troubles cardio-vasculaires (L. & B. Wilken J. Inher. Metab. Dis. 1997). Ainsi les

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5 patients souffrant d'homocystéinurie, une anomalie génétique, présentent de manière prématuée des troubles athérosclérosiques et thrombo-emboliques (SH. Mudd & al. The metabolic and molecular bases of inherited disease, 1995), ainsi que des maladies cardio-vasculaires (McCully . Atherosclerosis Rev 11, 1983). L'homocystéinurie est une déficience héréditaire dont la forme homozygote est rare. On estime que la prévalence de la forme hétérozygote est de 1 pour 200 dans la population générale.

10 L'homocystéinurie est due à des taux élevés d'homocystéine dans le plasma des patients atteints. L'administration de la bétaïne permet d'abaisser la concentration d'homocystéine dans le sang.

Dans la publication WO 95 / 157 50, L'auteur afin de prévenir les désordres vasculaires chez les patients homocystéinuriques propose l'usage dans ses ingrédients de la bétaïne.

15 La publication WO 98 / 19690 s'intéresse également aux patients souffrant d'un taux d'homocystéine élevé dans le sang. L'usage de la bétaïne entre autres ingrédients est destiné à réduire le taux d'homocystéine dans le sang, étant établi que l'homocystéine est un facteur positif de risque dans l'occurrence des maladies cardio-vasculaires, ainsi que dans la maladie d'Alzheimer.

20 La publication EPO 347 864 décrit l'usage de la bétaïne parmi ses ingrédients afin de lutter contre l'augmentation dans le plasma humain des groupes sulphydryls dûs à la cystéine ainsi qu'à l'homocystéine, et ainsi inhiber la formation des plaques d'athérosclérose.

25 Cette activité anti-athérosclérosique est connue et largement documentée. Ces publications s'intéressent à l'activité de la bétaïne sur le métabolisme des lipides (Zapadnyuk & al. Biol. Med. 1987) ainsi que sur celui du cholestérol (Panteleimonova & al . Farmakol. Toksikol , Moscow 1983).

30 La publication WO 97 38685 décrit l'utilisation de la bétaïne et de la taurine afin de traiter les complications résultant de l'ischémie dans certains organes. L'ischémie est un arrêt localisé de la circulation sanguine et ne représente qu'une des pathologies dues à la thrombose.

35 La publication EPO 781 554 décrit dans ses exemples l'expérimentation sur des coeurs énucléés, c'est à dire des coeurs extraits et isolés du système vasculaire. L'utilisation de la bétaïne pour ses propriétés osmoprotectrices et antiradicalaires connues permet ici aux auteurs de revendiquer son rôle protecteur du muscle cardiaque.

D'autres formes de bétaines ont été proposées (WO 97 / 06795) sans égaler à ce jour l'activité et les performances de la glycine bétaïne.

40 Aucune de ces publications ne dévoile l'activité de la glycine bétaïne vis à vis de la thrombose veineuse et/ou artérielle, ni son activité anti-agrégante et anticoagulante.

45 La glycine bétaïne dans le cadre de la présente invention peut être utilisée pour diverses applications cliniques telles que :

- Thromboses coronariennes et thromboses veineuses
- Infarctus, angine de poitrine, anévrisme, embolie pulmonaire, phlébite
- Les embolies cérébrales
- les chocs post-traumatiques d'origine chirurgicale ou non.
- La prévention des accidents de microcirculation dans les cas suivants : hémophilie, chimiothérapie, âge, contraception orale par les oestrogènes, obésité, tabagisme, prothèse.
- La prévention des risques liés à l'administration des produits de contraste ioniques et non ioniques.

MATERIEL ET METHODE

5

A/ Principe de la thrombose induite par laser.

(Seiffge D. et al., 1989 ; Weichter W. et al., 1983)

Dans ce modèle, la lésion de la paroi vasculaire est induite par un faisceau laser. Ce faisceau entraîne une lésion limitée de l'endothélium vasculaire (seulement 1 à 2 cellules sont détruites). La mise à nu du sous-endothélium, surface thrombogène, amène l'adhésion des plaquettes par l'intermédiaire de la glycoprotéine Ib. Cette adhésion des plaquettes est suivie par leur activation. Elles forment des pseudopodes et sécrètent le contenu de leurs granules. Cette activation entraîne l'apparition de la glycoprotéine IIb-IIIa nécessaire à l'agrégation des plaquettes entre elles. Cette lésion est induite au niveau de la microcirculation mésentérique du rat. Elle est immédiatement suivie par la formation d'un thrombus (quelques secondes). Ce thrombus qui grossit rapidement, sous l'effet du flux sanguin, embolise avant de se former à nouveau.

20 Dans ce dispositif l'évaluation de l'effet de la glycine bétaine a été mené conjointement à l'étude de deux molécules pharmacologiquement actives utilisées comme référence: l'acide acétylsalicylique et l'héparine (de bas poids moléculaire). L'évaluation a également porté sur l'activité de la glycine bétaine par rapport aux effets prothrombotiques induits par les produits de contraste.

25

B/ Thrombose induite par stase.

Une laparotomie est effectuée pour dégager la veine cave inférieure sur laquelle on pratique une ligature à To, à To + 2h injection sous cutanée de la glycine bétaine, à To + 6h prélevement du caillot.

C/ Protocole expérimental.

Pour ces études on a utilisé des rats Wistar mâles. Leur poids est compris entre 200 et 250 grammes. Après une période de stabulation de 8 jours, les rats sont soumis à un jeûne de 12 heures. Ils sont ensuite anesthésiés, la glycine bétaine est administrée par voie sous cutanée et le mésentère (laser) ou la veine cave (stase) sont dégagés aux fins des expérimentations.

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EXEMPLES:

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Exemple 1 : Evaluation du nombre d'emboles et de la durée d'embolisation après altération vasculaire par les tirs lasers

	Nombre d'emboles	Durée d'embolisation (minutes)
Témoin négatif NaCl 0.9%	5,33 ± 0,58	2 ± 0
Glycine bétaine 5mg/kg	2 ± 0	1 ± 0
Acide acétylsalicylique 100mg/kg	1 ± 1	0,33 ± 0,58
Héparine 2 mg/kg	2,67 ± 0,58	1 ± 0

10

La glycine bétaine réduit d'une façon significative le nombre d'emboles et le temps d'embolisation après altération vasculaire par des tirs lasers. Ces résultats démontrent sa puissante activité anti-thrombotique.

15

Exemple 2 : Evaluation du temps d'hémorragie provoquée

(E. Dejana. Bleeding time in rats . Thrombosis. Rech. 1982)

20

	THP (secondes)
Témoin négatif NaCl 0.9%	101,52 ± 5,7
Glycine bétaine 5mg/kg	95 ± 5
Acide acétylsalicylique 100mg/kg	276,67 ± 20,82
Héparine 2 mg/kg	313,33 ± 20

Les résultats montrent que la glycine bétaine maintient le temps d'hémorragie provoquée dans les valeurs du témoin négatif. La glycine bétaine en plus de son activité anti-thrombotique n'engendre pas de risques hémorragiques comparativement aux témoins positifs.

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Exemple 3: Evaluation de l'agrégation plaquetttaire après altération vasculaire par les tirs lasers

5 (Cardinal & Flower. Pharmacol. Method. 1980)

	Amplitude (Ohms)	Vélocité (Ohms/min)
Témoin négatif NaCl 0.9%	13 ± 1	9 ± 1
Glycine bétaine 5mg/kg	0.66 ± 1.15	1.66 ± 1.15
Acide acétylsalicylique 100mg/kg	2,33 ± 2.08	2 ± 1
Héparine 2 mg/kg	4.33 ± 0.57	2.66 ± 0.50

Les résultats démontrent l'activité anti-agrégante de la glycine bétaine.

10

Exemple 4 : Evaluation de l'effet de la vis à vis des cellules sanguines
a/ Dénombrement des plaquettes

	Nombre de plaquettes (10^9)
Témoin négatif NaCl 0.9%	788 ± 30.14
Glycine bétaine 5mg/kg	804.67 ± 20.03
Acide acétylsalicylique 100mg/kg	855.33 ± 63.17
Héparine 2 mg/kg	777.33 ± 6.43

15

b/ Dénombrement des globules blancs

	Nombre des globules blancs (10^9)
Témoin négatif NaCl 0.9%	5.03 ± 0.20
Glycine bétaine 5mg/kg	4.43 ± 0.32
Acide acétylsalicylique 100mg/kg	4.33 ± 1.00
Héparine 2 mg/kg	5.80 ± 0.10

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c/ Dénombrement des globules rouges

5

	Nombre des globules rouges (10^{12})
Témoin négatif NaCl 0.9%	6.56 ± 0.15
Glycine bétaine 5mg/kg	6.19 ± 0.25
Acide acétylsalicylique 100mg/kg	6.15 ± 0.31
Héparine 2 mg/kg	6.20 ± 0.20

Le dénombrement des éléments figurés du sang reste dans les valeurs du témoin négatif et démontre l'innocuité de la glycine bétaine

10

Exemple 5 : Bilan biologique

a/ Temps de Quick

	TQ (secondes)
Témoin négatif NaCl 0.9%	17 ± 1
Glycine bétaine 5mg/kg	16.9 ± 1.05
Acide acétylsalicylique 100mg/kg	18.33 ± 2.08
Héparine 2 mg/kg	29.50 ± 0.52

15

b/ Temps de céphaline activée (TCA)

	TCA (secondes)
Témoin négatif NaCl 0.9%	20.5 ± 0.5
Glycine bétaine 5mg/kg	39.9 ± 1.05
Acide acétylsalicylique 100mg/kg	27.26 ± 1.1
Héparine 2 mg/kg	39.46 ± 1.36

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c/ Dosage du fibrinogène

5

	Fibrinogène (g/l)
Témoin négatif NaCl 0.9%	2.45 ± 0.19
Glycine bétaine 5mg/kg	1.7 ± 0.1
Acide acétylsalicylique 100mg/kg	2.19 ± 0.33
Héparine 2 mg/kg	2.13 ± 0.25

d/ Dosage de l'alpha.2-Antiplasmine (α2AP)

10

	α 2AP (%)
Témoin négatif NaCl 0.9%	30.16 ± 0.85
Glycine bétaine 5mg/kg	29.7 ± 0.68
Acide acétylsalicylique 100mg/kg	29.36 ± 0.92
Héparine 2 mg/kg	29.4 ± 1.01

e/ Dosage de l'Antithrombine III (AT III)

15

	AT III (%)
Témoin négatif NaCl 0.9%	86 ± 3
Glycine bétaine 5mg/kg	89.5 ± 1.37
Acide acétylsalicylique 100mg/kg	85.33 ± 3.51
Héparine 2 mg/kg	77.66 ± 1.52

Exemple 6: Evaluation de l'activité de la glycine bétaine en fonction du temps

5

Groupes expérimentaux : Le produit est testé à 5 mg/ kg
Thrombose induite par laser

	Contrôle	NaCl 0,9%
10	Groupe I	Le produit est injecté 1 heure avant l'expérimentation
	Groupe II	Le produit est injecté 2 heures avant l'expérimentation
	Groupe III	Le produit est injecté 24 heures avant l'expérimentation
	Groupe IV	Le produit est injecté 48 heures avant l'expérimentation

15

a) Effet du produit testé (5 mg/ml/kg) sur le Temps d'Hémorragie Provoquée.

Groupes	T.H.P (secondes)
NaCl 0,9 %	110 ± 21,2
I	105 ± 26,2
II	145 ± 15,52
III	115,5 ± 14,2
IV	120 ± 10,13

20

b) Effet du produit testé (5 mg/ml/kg) sur la thrombose artérielle induite par faisceau laser

Groupes	Nombre de tirs	Nombre d'emboles	durée d'embolisation (minutes)
NaCl 0,9 %	2,5 ± 0,84	5,7 ± 1,5	2,1 ± 0,69
I	3,49 ± 1,07	1,8 ± 1,44	0,51 ± 0,5
II	3,0 ± 1,5	1,4 ± 1,18	0,3 ± 0,23
III	2,50 ± 1,25	1,99 ± 0,4	1,00 ± 0,5
IV	2,7 ± 1,0	2,2 ± 0,69	1,5 ± 0,6

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→ ~~Effet du produit testé (Sanglyg) sur l'aggrégation plaquette-érythrocyte~~

Groupes	Amplitude (Ohms)	Vélocité (Ohms / minutes)
NaCl 0,9 %	24,23 ± 0,5	14,4 ± 2,3
I	11,33 ± 3,08	8,2 ± 0,2
II	13,2 ± 3,5	9,3 ± 1,8
III	12,7 ± 4,1	8,7 ± 1,3
IV	13 ± 2,8	8,7 ± 1,15

5

d) Evaluation de l'effet de la glycine bétaine sur les facteurs de coagulation après administration répétitive sur 5 jours de traitement.

	TCA (secondes)	Temps de Quick (secondes)	Fibrinogène g/l
Témoin non traité	21,25 ± 2,3	16,1 ± 1,0	3,03 ± 0,45
Glycine bétaine (5 mg / kg / j)	39,3 ± 2,3	19,8 ± 1,2	2,2 ± 0,1

10

Exemple 7 : Evaluation de l'effet de la glycine bétaine sur la thrombose veineuse induite par stase.

a) Effet de la glycine bétaine sur le poids du caillot

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	Poids du caillot (mg)
Témoin non traité	4,033 ± 2
Glycine bétaine (1mg/kg)	3,1 ± 0,4
Glycine bétaine (2,5 mg/kg)	1,63 ± 0,76
Glycine bétaine (5mg/kg)	0,76 ± 0,4

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d) Evaluation de l'effet de la glycine bétaine sur le plasminogène

	Plasminogène %
NaCl 0,9 %	2,7 ± 0,33
Glycine bétaine (5 mg / kg)	1,66 ± 0,58
Glycine bétaine (2,5 mg / kg)	2 ± 0,15
Glycine bétaine (1 mg / kg)	2,44 ± 0,58

5

c) Evaluation de l'effet de la glycine bétaine sur la coagulation

	TCA (secondes)	Temps de Quick (secondes)	Fibrinogène g / l
Témoin non traité	30,2 ± 2,7	16,1 ± 1,0	3,03 ± 0,45
Glycine bétaine (1 mg/kg)	29,1 ± 2,3	16,2 ± 1,2	2,63 ± 0,3
Glycine bétaine (2,5 mg/kg)	31,2 ± 2,6	16,6 ± 0,7	2,2 ± 0,17
Glycine bétaine (5 mg/kg)	33,5 ± 1,9	15,6 ± 0,4	2,32 ± 0,33

d) Evaluation de l'effet de la glycine bétaine sur les facteurs de coagulation

10

	Anti Xa unité / ml	Anti IIa unité / ml
Glycine bétaine (5 mg / kg)	0,35 ± 0,15	-
Glycine bétaine (2,5 mg / kg)	0,14 ± 0,10	-
Glycine bétaine (1 mg / kg)	0,08 ± 0,1	-

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~~Le traitement avec la glycine bétaine inhibe les complications thrombo-emboliques déclenchées par les firs lasers. En effet, ce traitement avec la glycine bétaine, avant les firs lasers diminue l'adhésion des plaquettes et leur agrégation au niveau vasculaire.~~

5 Le traitement avec la glycine bétaine inhibe les complications thrombo-emboliques. En effet, ce traitement avec la glycine bétaine, avant l'induction de la thrombose, a montré un haut potentiel antithrombotique au niveau de tous les paramètres entrant en jeu dans le processus de la formation du thrombus. De plus, les résultats des paramètres biologiques démontrent la 10 parfaite innocuité de la glycine bétaine qui contrairement aux produits de référence utilisés (aspirine et héparine), n'induit aucun effet hémorragique ni d'effet secondaire indésirable. Ces caractéristiques confèrent à la glycine bétaine, en plus de son efficacité démontrée, la particularité de pouvoir être administrée aux personnes à risque hémorragique ainsi qu'aux personnes qui présenteraient des risques de sensibilité ou d'allergie face aux traitements 15 antithrombotiques conventionnels (hémophiles, allergiques). La glycine bétaine ne provoquant de thrombopénies ou désordres hémorragiques (exemples 2 & 4). Le résultat expérimental de l'exemple 5, c, démontre une consommation du fibrinogène .

20 Il est à noter que, dans les mêmes conditions expérimentales, pour la conservation du sang la glycine bétaine est apparue comme possédant un haut pouvoir anti-coagulant comparativement à des tubes héparinés ou contenant de l'E.D.T.A. Les doses actives de glycine bétaine sont apparues entre 3 et 5 mg par tube à hémolyse. Ce résultat expérimental démontre un haut potentiel anticoagulant de la glycine bétaine. L'utilisation de la glycine bétaine comme anticoagulant peut être revendiquée, tant pour le traitement du corps humain *in vivo*, que pour la conservation du sang *ex vivo*. 25

Evaluation de l'activité de la glycine bétaine vis à vis des produits de contraste.

30 Dans le cadre de la recherche sur les effets anti-thrombotiques et dans le souci de compléter l'approche de l'efficacité de la glycine bétaine, nous avons évalué l'effet de la glycine bétaine sur l'augmentation des risques thrombo-emboliques liés à l'utilisation des produits de contraste connus pour leurs pouvoirs prothrombotiques. L'intérêt représenté par l'utilisation de ce modèle est qu'il permet l'observation directe de la formation du thrombus au site de la lésion vasculaire. Ces résultats expliquent l'apparition d'occlusions thrombotiques 35 lors des angioplasties, surtout chez des patients, dont l'endothélium est déjà endommagé ou lésé. L'angioplastie coronaire cause une dénudation de l'endothélium, exposant le collagène, l'élastine et les cellules musculaires lisses au sang circulant, en analogie avec le modèle de thrombose expérimentale utilisé. Ainsi, l'apparition de nouveaux thrombi est plus élevée chez des patients présentant un infarctus du myocarde récent ou une plaque coronaire excentrique.

40 L'administration des produits de contraste, diminue le nombre de globules blancs, le nombre de globules rouges et le nombre de plaquettes. Les produits de contraste interagissent avec les leucocytes, induisent la libération de leukotriènes, augmentent la perméabilité vasculaire et exercent un effet chimiotactique. De plus, les produits de contraste agissent sur le contrôle de l'expression de la P-selectine et provoquent l'adhésion des globules blancs à l'endothélium vasculaire . Il a été démontré que l'utilisation des produits de contraste était 45 associée avec l'apparition de thrombi d'importance variable en fonction du produit utilisé.

Deux produits de contrastes ont été étudiés : Hexabrix® (ionique) et Iopamidol ® (non ionique)

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~~Exemple 8 : Evaluation du nombre d'emboles et de la durée d'embolisation après altération vasculaire par les tirs lasers et administration des produits de contrastes.~~

	Nombre d'emboles	Durée d'embolisation (minutes)
Témoin négatif NaCl 0.9%	5.33 ± 0.58	2 ± 0
Hexabrix®	8 ± 1	3.67 ± 0.58
Iopamidol®	11.67 ± 0.50	6.33 ± 0,52
Glycine bétaine 5mg/kg + Hexabrix®	4 ± 1	2 ± 0
Glycine bétaine 5mg/kg + Iopamidol®	5.33 ± 0.58	2.33 ± 0.48

5

Exemple 9 : Evaluation du temps d'hémorragie provoquée (THP)

	THP (secondes)
Témoin négatif NaCl 0.9%	101.52 ± 5.7
Hexabrix®	195 ± 13.23
Iopamidol®	128 ± 7.64
Glycine bétaine 5mg/kg + Hexabrix®	150 ± 5
Glycine bétaine 5mg/kg + Iopamidol®	111 ± 6.60

10 **Exemple 10: Evaluation de l'agrégation plaquettaire après altération vasculaire par les tirs lasers**

	Amplitude (Ohms)	Vélocité (Ohms/min)
Témoin négatif NaCl 0.9%	13 ± 1	9 ± 1
Hexabrix®	6 ± 1	5.66 ± 0.57
Iopamidol®	15 ± 2.64	12.33 ± 0,50
Glycine bétaine 5mg/kg + Hexabrix®	2 ± 1	5 ± 0
Glycine bétaine 5mg/kg + Iopamidol®	4.66 ± 0.52	9.33 ± 0.8

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~~Exemple 11 : Evaluation de l'effet de la glycine bétaine vis à vis des cellules sanguines~~

5

a/ Dénombrement des plaquettes

	Nombre plaquettes (10^9)
Témoin négatif NaCl 0.9%	788.33 ± 30.14
Hexabrix®	620 ± 10
Iopamidol®	585.67 ± 23.54
Glycine bétaine 5mg/kg + Hexabrix®	669.67 ± 7.37
Glycine bétaine 5mg/kg + Iopamidol®	704.33 ± 92.33

b/ Dénombrement des globules blancs

	Nombre globules blancs (10^{12})
Témoin négatif NaCl 0.9%	5.03 ± 0.20
Hexabrix®	2.96 ± 0.21
Iopamidol®	3.06 ± 0.35
Glycine bétaine 5mg/kg + Hexabrix®	4.20 ± 0.1
Glycine bétaine 5mg/kg + Iopamidol®	3.9 ± 0.3

10

c/ Dénombrement des globules rouges

	Nombre globules rouges (10^9)
Témoin négatif NaCl 0.9%	6.56 ± 0.15
Hexabrix®	5.43 ± 0.47
Iopamidol®	5.5 ± 0.36
Glycine bétaine 5mg/kg + Hexabrix®	6.5 ± 0.15
Glycine bétaine 5mg/kg + Iopamidol®	6.6 ± 0.19

-15-

~~Champagne 12 - Biomarckage~~

a/ Temps de Quick

5

	TQ (secondes)
Témoin négatif NaCl 0.9%	17 ± 1
Hexabrix®	24.13 ± 1
Iopamidol®	28.1 ± 0.75
Glycine bétaine 5mg/kg + Hexabrix®	16.36 ± 0.56
Glycine bétaine 5mg/kg + Iopamidol®	17.83 ± 1.2

b/ Temps de céphaline activé (TCA)

	TCA (secondes)
Témoin négatif NaCl 0.9%	20.5 ± 0.5
Hexabrix®	49.3 ± 1.85
Iopamidol®	41.33 ± 0.8
Glycine bétaine 5mg/kg + Hexabrix®	25.4 ± 0.61
Glycine bétaine 5mg/kg + Iopamidol®	22.4 ± 0.7

10

c/ Dosage du fibrinogène

	Fibrinogène (g/l)
Témoin négatif NaCl 0.9%	2.45 ± 0.19
Hexabrix®	1.49 ± 0.18
Iopamidol®	1.5 ± 0.8
Glycine bétaine 5mg/kg + Hexabrix®	1.7 ± 0.09
Glycine bétaine 5mg/kg + Iopamidol®	1.9 ± 0.1

- 16 -

d/ Dosage de l'alpha.2-Antiplasmine (α 2AP)

5

	α 2AP (%)
Témoin négatif NaCl 0.9%	30.16 ± 0.85
Hexabrix®	23.26 ± 1.06
Iopamidol®	25.23 ± 0.95
Glycine bétaine 5mg/kg + Hexabrix®	25.66 ± 0.64
Glycine bétaine 5mg/kg + Iopamidol®	28.13 ± 0.8

e/ Dosage de l'Antithrombine III (AT III)

10

	AT III (%)
Témoin négatif NaCl 0.9%	86.3 ± 3
Hexabrix®	81.63 ± 0.66
Iopamidol®	70.6 ± 1.51
Glycine bétaine 5mg/kg + Hexabrix®	79.1 ± 1.05
Glycine bétaine 5mg/kg + Iopamidol®	87.26 ± 0.9

Le traitement avec la glycine bétaine inhibe les complications thrombo-emboliques

15 associées à l'utilisation des produits de contraste. En effet, ce traitement avec la glycine bétaine, avant ou pendant l'injection des produits de contraste, diminue l'adhésion des plaquettes et leur agrégation au niveau vasculaire. Ces résultats démontrent l'effet anti-thrombotique et thrombolytique de la glycine bétaine. Il est à noter que les produits de contraste peuvent avoir d'autres effets secondaires telle que la stase sanguine au niveau des cathéters et les lésions endothéliales causées par les procédures d'administrations elles-mêmes. La glycine bétaine remédie à ces effets indésirables.

5 CONCLUSION

La glycine bétaine possède les mêmes, voire de meilleures, caractéristiques thérapeutiques que les anticoagulants et les anti-agrégants étudiés (acide acétylsalicylique et l'héparine), tout en ne présentant aucun effet indésirable.

10 Les performances supérieures en terme d'efficacité thérapeutique de la glycine bétaine par rapport à ces deux molécules (acide acétylsalicylique et héparine) incitent à la formulation d'un médicament ayant pour principe thérapeutiquement actif la glycine bétaine. Ce médicament étant destiné au traitement des thromboses et des affections thrombo-emboliques.

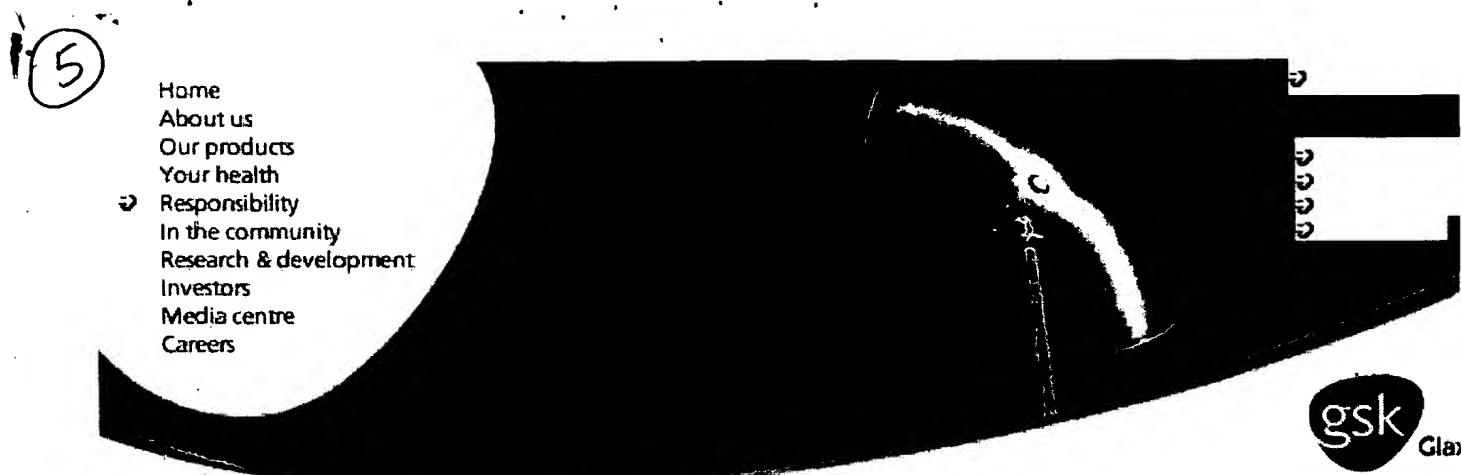
15 Selon les résultats exposés ce médicament revendique aussi les indications anticoagulant, anti-agrégant et fibrinolytique. L'innocuité démontrée de cette molécule permet d'envisager des traitements à long terme sans pour autant nécessiter une surveillance biologique.

20 L'intérêt de l'utilisation de la glycine bétaine réside dans le fait qu'elle agit à plusieurs niveaux de l'hémostase à savoir au niveau de l'agrégation plaquettaire, au niveau de la coagulation et au niveau fibrinolytique. Cette activité est durable et évite des administrations répétitives ce qui constitue une amélioration notable par rapport aux traitements existant. 25 L'administration de la bétaine n'induit aucun risque hémorragique, ni autres effets secondaires (ex : thrombopénie induite à l'héparine) ce qui constitue une avancée majeure en thérapeutique antithrombotique.

REVENDICATIONS

5

1. Utilisation de la glycine bétaine pour l'obtention d'un médicament destiné à traiter les thromboses non induites par l'homocystéinurie.
2. Utilisation de la glycine bétaine pour l'obtention d'un médicament destiné à traiter les affections thrombo-emboliques non induites par l'homocystéinurie.
- 10 3. Utilisation de la glycine bétaine pour l'obtention d'un médicament destiné à traiter les désordres de la coagulation du sang chez un patient.
4. Utilisation de la glycine bétaine pour l'obtention d'un médicament destiné à traiter les désordres de l'agrégation plaquettaire chez un patient.
- 5 Utilisation de la glycine bétaine pour l'obtention d'un médicament destiné à lyser un thrombus.
- 15 6. Utilisation de la glycine bétaine pour l'obtention d'un médicament destiné à traiter les thromboses chez les sujets à risque hémorragique.
7. Utilisation de la glycine bétaine comme agent anticoagulant pour la conservation du sang ex-vivo.
- 20 8. Utilisation de la glycine bétaine pour l'obtention d'un médicament destiné à contrecarrer les effets thrombo-emboliques induits par les produits de contraste.
9. Utilisation de la glycine bétaine selon la revendication 8, caractérisée en ce qu'elle puisse être simultanément administrée avec des produits de contraste à un patient.
- 25 10. Utilisation de la glycine bétaine comme principe actif selon l'une quelconque des revendications 1 à 9, caractérisée en ce qu'une quantité efficace de glycine bétaine est associée à un support, véhicule ou excipient pharmaceutiquement acceptable.



Responsibility

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Healthy volunteer studies

Before a pharmaceutical company can initiate the evaluation of a new medicinal compound in humans, it must first conduct extensive preclinical or laboratory research. This research typically involves years of experiments including animals and human cells. If this stage of testing is successful, a pharmaceutical company provides these data to regulatory authorities, requesting approval to begin evaluating the drug in humans. This evaluation is done through clinical trials and is conducted in three main phases. Each phase addresses different questions that determine if the drug can proceed to the next phase.

It is an ethical requirement that participants in clinical trials voluntarily decide to take part after having been provided with understandable information about the study. In most studies the participants will be patients with the disease or condition for which the new medicine is being evaluated. In the earliest phase of human trials (Phase I) when only the basic characteristics of the new medicine are being assessed, the volunteers normally do not have the disease or condition, ie they are healthy volunteers.

Phase I studies are primarily concerned with assessing the drug's safety in humans. An attempt is made to establish the dose range tolerated by volunteers for single and for multiple doses. These studies are designed to determine what happens to the drug in the human body - how it is absorbed, metabolised, and excreted. This initial phase of testing in humans is usually conducted in a small number of healthy volunteers (20 to 100), who are reimbursed for their time and discomfort and based on both the study design and the trends/practices for compensation in the local area. Sometimes these studies are conducted in patients (eg, cancer studies) and this may include research into how the disease may impact the way in which the drug is handled in the human body.

An Ethics Committee (EC) or Institutional Review Board (IRB) approval is required to ensure that appropriate safeguards are in place to protect the rights and welfare of research subjects.

This initial phase of testing typically takes several months. About 70 per cent of experimental drugs pass this initial phase of testing.

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Research & de Clinical Trial Re

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Diet Pills Glossary of Weight Loss Terms

Adipex - A Brand Name for Phentermine, an appetite suppressant used to reduce calorie intake.

Adipocytes - The scientific term for Fat Cells, being various types of specialized cells found in adipose tissue used for fat storage. One might say this is a rather obese term for Fat Cells. ;-)

Adipose - The fat found in Adipose tissue.

Adipose tissue - A specialized type of tissue for storing cellular fat.

Amino acids - The essential building blocks of Proteins, nine of which cannot be manufactured by the body and therefore have to be obtained through food intake.

Anorectic drugs - Pharmaceutical drugs designed as appetite suppressants to reduce calorie intake.

Anorexia Nervosa - A psychophysiological disorder characterized by an abnormal fear of becoming obese and therefore a distorted self-image, resulting in an unwillingness to eat leading to severe weight loss. It can be accompanied by vomiting, excessive exercise and other physiological changes.

Bariatric - The branch of medicine dealing with the causes, prevention and treatment of obesity, both pharmacological and surgical.

Binge Eating Disorder - An eating disorder involving uncontrolled eating of large amounts of food but without vomiting or laxative purging.

Body Mass Index (BMI) - A calculation used by researchers and physicians to estimate the ratio of your body weight to your height, thus estimating the degree by which you are over or underweight and thus the fat load on your body.

Bontril SR - An appetite suppressant that works by stimulating the nervous system.

Bulimia Nervosa - An eating disorder involving episodic binge eating followed by feelings of guilt, depression, and self-condemnation. It also involves measures to prevent weight gain, such as self-induced vomiting.

[American Heart Association](#)

[Food and Drug Administration](#)

[Nutrition and Health!](#)

[Phentermine Articles](#)

[Phentermine Fact List](#)

[Weight Control Information Network](#)

[BMI Calculator](#)
[Diet Pills Glossary](#)
[Mexican Pharmacy](#)

excessive laxatives, dieting, or fasting.

Calories - A unit of measurement for the amount of energy that is released from food upon oxidation by the body. Also the amount of heat required to raise the temperature of 1 kilogram of water by 1°C at 1 atmosphere pressure.

Carbohydrates - A group of organic compounds, including sugars, starch and fiber, that is a major source of energy for animals.

Cellulite - The dimples and bumps in the skin, usually around the thighs and buttocks, caused when the natural structure of the skin is stretched by fat cells growing too large.

Compulsive Overeating - Also known as Binge Eating.

Didrex - An appetite suppressant that works by stimulating the nervous system.

Diet - The food and drink a person or animal consumes in their normal diet and a regulated eating plan for medical reasons or as a measure to promote weight loss.

Diuretics - A drug that increases the discharge of urine, used to move water through the body quickly as a form of purging..

Exercise - A physical or mental activity used as a method of maintaining and improving a level of fitness. An important part of an overall weight loss plan of action.

Fad Diets - Fashionable or Trendy diets that may or may not actually help in reducing weight. These diets should be used with caution and a professional medical opinion sought.

Fastin - A brand name for Phentermine previously manufactured by : Glaxo Kline Beecham but no longer available.

Fat - Animal tissue containing glycerol and fatty acids. Also used to describe someone who has too much fat and is therefore plump or obese.

FDA - The Food & Drug Administration is the US Federal agency responsible for the regulation of biotechnology food products.

Generic Drug - A drug whose patent has expired thus enabling it to be manufactured by any company. Phentermine is an example of a Generic Drug.

Health - The overall condition of an organism at a given time in regard to soundness of body or mind and freedom from disease or abnormality.

Meridia (sibutramine) - A weight loss diet pill that suppresses appetite by inhibiting the re-uptake of applicable hormones.

Metabolism - The chemical processes that take place within a living organism that break down substances to provide energy and/or materials which are then re-synthesized into new and necessary substances to support life.

Nutraceutical - A naturally occurring food (Garlic, Soy) or food supplement (Cod Liver Oil) believed to have beneficial effects on human health.

Nutritionist - An expert trained in the field of nutrition who is able to give advice in regard to allergies or health problems, and plan healthy diets to assist in weight loss.

Obese, Obesity - The condition having and increased body weight caused by excessive accumulation of fat. This is usually indicated as having a Body Mass Index in excess of 30.

Overweight - The condition of weighing more than is normal or healthy for one's age or build. This is usually indicated as having a Body Mass Index higher than 25 but lower than 30.

Phentermine - An appetite suppressant that disrupts the transmission of signals from the neurotransmitters and is used in the management of obesity.

Proteins - A group of complex organic macromolecules that are the building blocks of all living cells and are therefore essential in the diet of animals for the growth and repair of tissue.

Saturated Fats - Fatty acids that are saturated with Hydrogen atoms; mainly found in animal tissue, and should be restricted in the diet.

Simple Sugars - Single Molecule sugars such as glucose, fructose and galactose.

Sucrose - A crystalline form of fructose and glucose found in many fruits and extracted as ordinary table sugar.

Tenuate - An Appetite Suppressant that works by stimulating the central nervous system, increasing heart rate and blood pressure and decreasing your appetite.

Xenical (Orlistat) - A Diet Drug that works in the digestive system by blocking about one-third of the fat you eat from being digested.

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We strongly advise you consult your doctor on a regular basis.





Back to: [Clinical Center Home Page](#) > [Participate in Clinical Studies](#)

FAQs About Clinical Studies

If you are in the process of learning about clinical trials or are considering participating in one, you may be interested in looking at the [Patient Recruitment Research \(pdf\)](#) (Requires Adobe's free [Acrobat Reader](#)), which describes the role of a patient in clinical research. In addition anyone with questions to call the Patient Recruitment Office at 1-800-411-1222. You may also want to try the ["Topics A-Z"](#) to get an alphabetical index to all visitor- and patient-related subject areas.

1. What are clinical studies?

Clinical studies are research studies in which real people participate as volunteers. Clinical research studies (sometimes called protocols) are a means of developing new treatments and medications for diseases and conditions. There are strict rules for clinical trials, which are monitored by the National Institutes of Health and the U.S. Food and Drug Administration. Some research studies at the Clinical Center involve promising new treatments that may directly benefit patients.

2. Why should I participate?

The health of millions has been improved because of advances in science and technology, and the willingness of the individuals like you to take part in clinical research. The role of volunteer subjects as partners in clinical research is critical to the quest for knowledge that will improve the health of future generations. Without your help, the research studies at the Clinical Center cannot be accomplished.

3. What is a "healthy volunteer"?

A volunteer subject with no known significant health problems who participates in research to test a new drug, device, or procedure is known as a "healthy volunteer" or "Clinical Research Volunteer." The clinical research volunteer may be a member of the general public, a member of the community, an NIH investigator or other employee, or family members of a patient volunteer. Research procedures and treatments used in clinical research studies are designed to develop new knowledge, not to provide direct benefit to study participants. Clinical research studies have always played a vital role in medical research. We need to study healthy volunteers for several reasons: When we are testing a new technique such as a blood test or imaging device, we need clinical research volunteers to help us define the limits of the test. These volunteers are recruited to serve as controls for patient groups. They are often matched to patients on such characteristics as age, gender, or family relationship. They are then given the same test, procedure, or drug that the patient group receives. This allows us to learn about the disease process by comparing the patient group to the clinical research volunteers.

4. What are Phase I, Phase II and Phase III studies?

The phase 1 study is used to learn the "maximum tolerated dose" of a drug that does not produce unacceptable side effects. In this study, volunteers are followed primarily for side effects, and not for how the drug affects their disease. The first few volunteers receive low doses of the trial drug to see how the drug is tolerated and to learn how it acts in the body. The next group of volunteers receives larger amounts. Phase 1 studies typically offer little or no benefit to the volunteer subjects.

The phase 2 study involves a drug whose dose and side effects are well known. Many more volunteer subjects are involved in this study to learn about the drug's side effects, learn how it is used in the body, and learn how it helps the condition under study. Some volunteer subjects receive a placebo from a phase 2 study.

The phase 3 study compares the new drug against a commonly used drug. Some volunteer subjects will be given the new drug and some the commonly used drug. The trial is designed to find where the new drug fits in managing a particular condition. Determining the true benefit of a drug in a clinical trial is difficult. Determining the true benefit of a drug in a clinical trial is difficult.

5. What is a placebo?

Placebos are harmless, inactive substances made to look like the real medicine used in the clinical trial. Placebos allow investigators to learn whether the medicine being given works better or no better than ordinary treatment. In many studies, subjects are assigned to successive time periods, with either the placebo or the real medicine. In order not to introduce bias, the patient, and the study staff, are not told when or what the changes are. If a placebo is part of a study, you will always be informed in the consent form before you agree to take part in the study. When you read the consent form, be sure that you understand what approach is being used in the study you are entering.

6. What is the placebo effect?

Medical research is dogged by the *placebo effect* - the real or apparent improvement in a patient's condition due to what the investigator or the patient. Medical techniques use three ways to rid clinical trials of this problem. These methods discredit some previously accepted treatments and validate new ones. Methods used are the following: randomization, double-blind studies, and the use of a placebo.

7. What is randomization?

Randomization is when two or more alternative treatments are selected by chance, not by choice. The treatment chosen is based on the highest level of professional care and expertise, and the results of each treatment are compared. Analyses are done during a trial, which may last years. As soon as one treatment is found to be definitely superior, the trial is stopped. If the fewest number of patients receive the less beneficial treatment.

8. What are single-blind and double-blind studies?

In single- or double-blind studies, the participants don't know which medicine is being used, and they can describe what they are taking without bias. Blind studies are designed to prevent anyone (doctors, nurses, or patients) from influencing the results. This allows for scientifically accurate conclusions. In single-blind ("single-masked") studies, only the patient is not told what is being taken. In a double-blind study, only the pharmacist knows; the doctors, nurses, patients, and other health care staff are not informed. This is medically necessary, however, it is always possible to find out what the patient is taking.

9. Are there risks involved in participating in clinical research?

Risks are involved in clinical research, as in routine medical care and activities of daily living. In thinking about the risks involved, it is helpful to focus on two things: the degree of harm that could result from taking part in the study, and the chance of the harm occurring. Most clinical studies pose risks of minor discomfort, lasting only a short time. Some volunteer subjects, however, experience complications that require medical attention. The specific risks associated with any research protocol are described in the consent document, which you are asked to sign before taking part in research. In addition, the major risk involved in a study will be explained to you by a member of the research team, who will answer your questions about the study. If you are deciding to participate, you should carefully weigh these risks. Although you may not receive any direct benefit as a result of participating in research, the knowledge developed may help others.

10. What safeguards are there to protect participants in clinical research?

The following section describes safeguards that protect the safety and rights of volunteer subjects. These safeguards include:

- The Protocol Review Process
- Informed Consent Procedures
- The Patient Representative
- The Patient Bill of Rights

Protocol review. As in any medical research facility, all new protocols produced at NIH must be approved by an institutional Review Board (IRB) before they can begin. The IRB, which consists of medical specialists, statisticians, nurses, social workers, and ethicists, is the advocate of the volunteer subject. The IRB will only approve protocols that address medically important issues in a scientific and responsible manner.

Informed consent. Your participation in any Clinical Center research protocol is voluntary. For every study in which you participate, you will receive a document called "Consent to Participate in a Clinical Research Study" that explains the protocol in straightforward language. A member of the research team will discuss the protocol with you, explain its details, and answer your questions. Reading and understanding the protocol is your responsibility. You may discuss the protocol with family and friends, but you will not be hurried into making a decision, and you will be asked to sign the document only after you understand the protocol and agree to the commitment. At any time after signing the protocol, you are free to change your mind and choose not to participate further. This means that you are free to withdraw from the study completely, or to refuse particular treatments. Sometimes, however, this will make you ineligible to continue the study. If you are no longer eligible or no longer wish to participate, you will return to the care of the doctor who referred you to NIH.

Patient representative. The Patient Representative acts as a link between the patient and the hospital. The Patient Representative makes every effort to assure that patients are informed of their rights and responsibilities, and that they understand what the Clinical Center is, what it can offer, and how it operates. We realize that this setting is unique and may generate questions at any time in the research process. As in any large and complex system, communication can be a problem and misunderstandings can occur. If any patient has an unanswered question or feels there is a problem they would like to discuss, they can call the Patient Representative.

Bill of Rights. Finally, whether you are a clinical research or a patient volunteer subject, you are protected by the Clinical Center's Bill of Rights. This document is adapted from the one made by the American Hospital Association for use in hospitals across the country. The bill of rights concerns the care you receive, privacy, confidentiality, and access to medical records.

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National Institutes of Health



Department of Health and Human Services

Phase 1 Clinical Studies

Phase 1 includes the initial introduction of an investigational new drug into humans. These studies are closely monitored and may be conducted in patients, but are usually conducted in healthy volunteer subjects. These studies are designed to determine the metabolic and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness. During Phase 1, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid, Phase 2 studies.

Phase 1 studies also evaluate drug metabolism, structure-activity relationships, and the mechanism of action in humans. These studies also determine which investigational drugs are used as research tools to explore biological phenomena or disease processes. The total number of subjects included in Phase 1 studies varies with the drug, but is generally in the range of twenty to eighty.

In Phase 1 studies, CDER can impose a clinical hold (i.e., prohibit the study from proceeding or stop a trial that has started) for reasons of safety, or because of a sponsor's failure to accurately disclose the risk of study to investigators. Although CDER routinely provides advice in such cases, investigators may choose to ignore any advice regarding the design of Phase 1 studies in areas other than patient safety.

US FDA draft about healthy Volunteer

9

FAQs on GCP ::

Clinical Trial Management | Ethics Committee | Informed Consent | Serious Adverse Event

CLINICAL TRIAL MANAGEMENT

1 How do we select a central laboratory?

Central Laboratory Services are a paramount component of a clinical trial. Although there is no regulatory requirement for central laboratory, the requirements of ICH GCP (data quality, documentation of procedures and document control) mean that central laboratory facilities are useful as part of integrated clinical trial procedures.

The criteria for selecting central laboratory are:

- Adherence to Good Laboratory Practice (GLP)
- Accreditation by a reputed international organization e.g. College of American Pathologists (CAP)
- Accreditation in India by National Accreditation Board for Testing and Calibration Laboratories (NABL)
- Quality assurance
- Staff training
- Efficiency of processing samples
- Speed of services
- Networking and transport of samples
- Past track record/ experience in a clinical trial.

2 What is the role of Independent Data-Monitoring Committee (IDMC) in a trial?

As per the ICH-GCP guidelines, the definition of Independent Data-Monitoring Committee (IDMC) (Data and Safety Monitoring Board, Monitoring Committee, Data Monitoring Committee) is as follows: An independent data-monitoring committee that may be established by the sponsor to assess at intervals the progress of a clinical trial, the safety data, and the critical efficacy endpoints, and to recommend to the sponsor whether to continue, modify or stop a trial. US FDA has developed "Guidance for Clinical Trial Sponsors On the Establishment and Operation of Clinical Trial Data Monitoring Committees".

The increasing use of IDMCs in industry-sponsored trials is due to several factors, including:

- the growing number of industry-sponsored trials with mortality or major morbidity endpoints;
- the increasing collaboration between industry and government in sponsoring major clinical trials, resulting in industry trials performed under the policies of government funding agencies, which often require IDMCs;
- heightened awareness within the scientific community of problems in clinical trial conduct and

analysis that might lead to inaccurate and/ or biased results, especially when early termination for efficacy is a possibility, and demand for approaches to protect against such problems.

All clinical trials do not require monitoring by a formal committee external to the trial organizers and investigators. IDMCs have generally been established for large, randomized multi-site studies that evaluate interventions intended to prolong life or reduce risk of a major adverse health outcome such as a cardiovascular event or recurrence of cancer.

Because monitoring of accumulating results is almost always essential in such trials, IDMCs should be established for controlled trials with mortality or major morbidity as a primary or secondary endpoint.

They may also be helpful in settings where trial participants may be at elevated risk of such outcomes even if the study intervention addresses lesser outcomes such as relief of symptoms.

Although IDMCs may prove valuable in other settings as well, an IDMC is not needed or advised for every clinical study.

3 In a multi-centric and centrally randomized phase III trial, can a patient enrolled in one of the sites be transferred to another site in the middle of a trial realizing a clear cut geographical advantage for the patient? As far as treatment is concerned, patient can get similar treatment from other site. What are the implications from GCP angle? Is it a practice that referral centers transfer patients in the middle of a trial to the regional centers very often?

There are several implications, which are discussed below: Ethics Committee (EC) of the site where patient was first enrolled (site 1) is responsible for overseeing the safety of the patient. If the patient is transferred to another site (site 2) you will need to inform EC of site 1 about the transfer of the patient. The EC can ask the reasons for the transfer and you will need their written agreement/ approval for this action. You will also need approval of EC of site 2, as they will have to take responsibility of the transferred patient's safety. This process is likely to take quite sometime as EC usually meets as per their schedule.

This means that the patient has to continue the follow up visits at site 1 till both ECs have given their agreement/ approval. This will also apply to SAE reporting.

If this practice is to be followed for all patients with geographical advantage, you will have to amend the protocol & obtain EC and Regulatory approvals.

Drug accountability and Randomization: If the trial is centrally randomized, it would be difficult to manage randomization, drug supplies and drug accountability at site 1 and 2. Financial payment to investigators for the patient and source data verification at 2 sites will be other major issues. However, the most critical issue is viewpoint of auditors and Regulatory inspectors. There is an US FDA warning letter (Sep 27, 2000) issued to an investigator (Courtesy: Dr Tanuja Kulshreshtha Amdavad).

The relevant excerpt is given below:

Dr Jeffery R Levenson, MD. The Research Consortium Inc., 9303 Seminole Boulevard Florida.

Page 4 under "summary of violations related to requirements for investigator reporting to IRB (21 CFR 312.66)"

You did not report to IRB changes in the research activity and unanticipated problems involving risks to human subjects or others, and made changes in the research without IRB approval for example:

You did not obtain IRB approval for continuing study based activities at neighboring hospital or nursing homes (secondary institutions) to which subjects were transferred after their enrolment at the --- for the

— general hospital —. We note that you did not seek IRB approval for continuing research at secondary institution even after IRB specifically informed you that they could not continue to serve as the IRB to the second institution. Instead you incorrectly informed the IRB that secondary institutions were only providing "incidental care" and were not research sites, when in fact you were conducting research on subjects and collecting data at these institutions."

In view of the above issues, transfer of patients in middle of the trial from site 1 to 2 is unlikely to be a common practice. If geographical location is an issue, the patient should be referred to the Centre in other city before screening, as the address would be known at that time!

4 Can the Principal Investigator delegate the duty of measuring blood pressure and recording on the source notes to a study team member who is not a physician?

Yes. If he/ she can demonstrate and document, that the person who is given the task has received such a training. Nurses do take BP in intensive care units and hospitals. If BP is an end point for efficacy/ safety, it is advisable to delegate this function to a physician. Even for bioequivalence studies, some sponsors insist that a physician should measure pulse and BP.

5 If one were using the traditional method of randomization (based on randomization list generated by data management and not IVRS) and If there are two requests for randomization from the investigator almost at the same time (within a gap of 1 min), but the date of consent of the subjects to the trial are different and they have been found eligible after screening on the same day then how should one go about randomizing the subject?

Although the dates of consent are different, as the randomization is on the same day one would first randomize the patient, whose request came 1 min earlier.

6 During the audit, how do you select the sample number of case report forms (CRFs) for source data verification? How much time is required for an audit?

There does not appear to be a consistent approach to CRF selection within the industry as different methods are being used by the companies. Some use square root of number of CRFs plus one (usually using total number of enrolled subjects - this can either include or exclude screened subjects depending on the company). Some define a minimum range, for example 3-5 CRFs.

Some companies' do 100 per cent Source Data Verification (SDV) for consent forms and then 100 per cent of SDV for 10 per cent of total CRFs. At the time of report audit, all primary efficacy data and adverse events would be audited 100 per cent for all patients randomized and/ or enrolled. Some companies only allow one day for an audit for one auditor, and in this case it would be difficult to review more than three CRFs in adequate detail unless the study was very simple.

If there are particular problems then it might be necessary to extend the review to more CRFs, or if there are so many discrepancies on the first two CRFs that there is no point reviewing any more as the recommendation would be for someone to re-monitor all CRFs.

7 Is it necessary that the Investigator should be a clinician? Can a Para-clinical person become an investigator?

Indian GCP guidelines insist that the investigator should be a medical person. Indian GCP 3.3.1.

Qualifications: The investigator should be qualified by education, training and experience to assume responsibility for the proper conduct of the study and should have qualifications prescribed by the Medical Council of India (MCI).

ICH-GCP does not insist on a particular medical qualification.

ICH GCP 4.1 Investigator's Qualifications and Agreements. 4.1.1 The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation requested by the sponsor, the IRB/IEC, and/or the regulatory authority (ies).

In case of bio-equivalence study, US FDA guidance (Compliance Program Guidance Manual For FDA Staff Chapter 48 Bioresearch Monitoring Human Drugs In-Vivo Bio-equivalence Compliance Program 7348.00) is as follows:

The clinical investigator in a bio-equivalence study is involved in the screening and dosing of human subjects, and will ordinarily be a physician. Ph. D clinical pharmacologists and Pharm. Ds are acceptable if a physician is available to cover medical emergencies.

8 For the phase I clinical trial who should be considered as healthy volunteer? Or what is the definition of a healthy volunteer?

US FDA guidance on General Considerations for Clinical Evaluation of Drugs discussed this issue. The term healthy suggests that volunteer should be free from abnormalities which could complicate interpretation of the experiment or which might increase the sensitivity of the volunteer to the toxic potential of the drug. Healthy volunteer is one who has no evidence of any clinical, biochemical or investigational abnormality (based on physical examination, lab investigations (hematology, liver function, renal function, blood sugar, cholesterol, triglycerides) and x-ray chest, ECG).

9 Kindly let me know whether there are any particular recommendations for the list of laboratory tests, which are to be performed in pre-study investigations for the volunteers? What are the criteria for inclusion of particular tests in pre-study/ post-study laboratory investigations? Is there any correlation between the study drug and inclusion of pre-study laboratory tests, apart from testing the patient's well being (inclusion criteria)?

US FDA guidance for the industry, "General Considerations for the clinical evaluation of drugs" suggests the following investigations for phase I volunteers:

- CBC including platelet, urinalysis, BUN or creatinine, liver function, FBS or 2 hour postprandial sugar, ECG.
- Any other investigations as per the profile of the drug under investigation.

For example, for a study on anti-platelet drug, it would be desirable to check BT, CT, PT in the volunteers. The importance of these tests is two fold. Volunteers should be free from abnormalities which would complicate the interpretation of experiment or which might increase the sensitivity of the subject to the toxic potential of the drug.

10 What is the situation of insurance and compensation in clinical trials in India?

Both ICH-GCP and Indian GCP provide guidelines on the issue: The compensation for trial related injury and claims are the essential items of informed consent form.

ICH-GCP 4.8.10: Both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the following:

- (j) The compensation and/ or treatment available to the subject in the event of trial-related injury.

Indian GCP 2.4.3.2. Essential information for prospective research on subjects

viii. free treatment for research related injury by the investigator/ institution;

ix. Compensation of subjects for disability or death resulting from such injury;

As regards insurance, ICH-GCP insists only if it is a regulatory requirement.

ICH-GCP 5.8 Compensation to Subjects and Investigators 5.8.1: If required by the applicable regulatory requirement(s), the sponsor should provide insurance or should indemnify (legal and financial coverage) the investigator/ the institution against claims arising from the trial, except for claims that arise from malpractice and/or negligence.

5.8.2 The sponsor's policies and procedures should address the costs of treatment of trial subjects in the event of trial-related injuries in accordance with the applicable regulatory requirement(s).

In contrast to ICH-GCP, Indian GCP is more explicit

Indian GCP 2.3.1.12. Finance and insurance

d. Study subjects should be satisfactorily insured against any injury caused by the study

e. The liability of the involved parties (investigator, sponsor/ manufacturer, institution(s) etc) must be clearly agreed and stated before the start of the study.

Indian GCP 2.4.7 also insists on compensation for accidental injury (temporary or permanent disability and death) and makes it obligatory for sponsor to provide compensation or insurance coverage. At present, it is difficult for a sponsor to obtain an insurance cover from Indian insurance companies. However, some general insurance companies are considering this. In absence of the insurance coverage, the pharmacy companies provide for indemnity to the investigator/ institute and agree to take care of medical costs for any trial related injury.

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Impact of the EU Directive on Ethical Review & Phase 1 Studies

An Institute/ABPI Joint Seminar

Liz Allen, Annelies Legters, Angie Major, Lesley Shelford & Lisa Tan

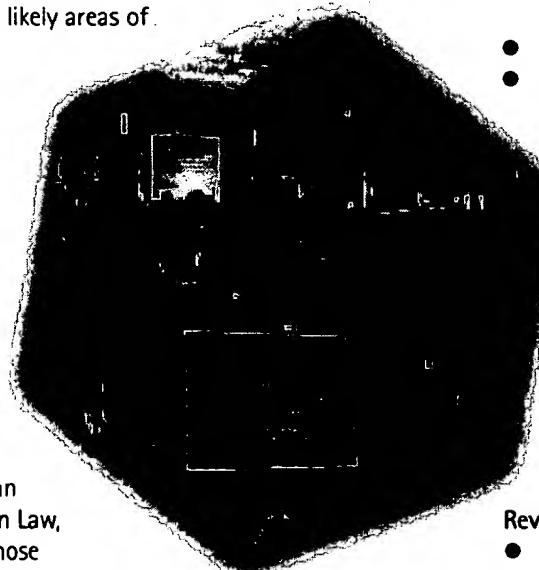
This two-day seminar was the first meeting to be jointly organised by the Institute of Clinical Research and the ABPI. The meeting was designed to investigate the impact of the EU Clinical Trials Directive on Ethics and Phase 1 clinical trials. Around 130 delegates attended, coming from the pharmaceutical industry, CROs, Academia and NHS & Independent Ethics Committees.

On the first morning, there were two parallel sessions:

- A Seminar For New Members of Ethics Committees dealing with the role, responsibilities and issues facing a new member of an Ethics Committee, structured protocol review and likely areas of difficulty
- A Workshop for all other attendees discussing The Implications of the EU Clinical Trials Directive and GAFREC for Phase 1 Ethics Committees

Seminar for new members

Kathy Doyle, Barrister and Senior Lecturer at Manchester Metropolitan University specialising in Law, Medicine and Ethics, whose presentation was entitled 'Being a member of an Ethics Committee', described the background of ethical regulation of research involving humans, citing numerous examples from the Nuremberg Trials, the development of the 10 principles of the Nuremberg Code, and The Declaration of Helsinki. However, as ethical violations continued further regulation and guidance was issued by The Royal College of Physicians (1984), The ABPI (1988), and of course ICH (1996). Ethical violation continues today and we must all continue to be vigilant.



There are a number of issues to which members of an Ethics Committee (EC) must pay attention:

Relating to the protocol

- Objectives or purposes (are they worthwhile, reasonable, and ethical, and do they meet a clinical need?)
- Study design (is the design scientific, valid and able to meet the requirements of the objectives?)
- Statistical issues (are there sufficient subject numbers to enable meaningful interpretation of the data, and is the proposed analysis valid for the study design?)
- Experience of research staff who are to perform the study
- Suitability of facilities
- Sufficiency of resources

For studies involving volunteers

- Recruitment procedures (relationship with investigators and/or employers)
- Payment of volunteers (potential for financial inducement)
- Informed Consent procedures (extent and format of information provided, how consent is obtained, indemnity, and compensation)
- Confidentiality
- Requirements of regulatory authorities

Reviewing pre-clinical toxicology data

- Are there any findings that cause concern?
- Is there the need to consult an independent expert?
- Is sufficient information available to enable first administration into man?
- What are the potential adverse effects?
- Were there any serious unanticipated and unexplained effects in animals?

Dr Michael Goggin presented 'A Structured Review of a Protocol in Medical Research', highlighting the major sections of a protocol (e.g. title, purpose, design, procedures, etc.) and the

issues to be considered within those sections. Ideally, protocols should be clear and unambiguous detailing accurately the objectives, design, variables to be measured, and analysis of the data. The protocol should be well thought through and reviewers must be satisfied that the protocol is acceptable in its present form. If it is not acceptable then it must be revised. In addition, to enable reviewers to adequately review a protocol, Dr Goggin suggested use of a protocol checklist to ensure that all the issues listed above had been considered.

Next on the agenda, Dr Chris Pick gave a short presentation to introduce delegates to the field of toxicology. Unfortunately, the authors were unable to attend this session.

Dr Frank Wells presented "Some Likely Areas of Difficulty", describing problems in the recruitment of healthy volunteers taking into account potential safeguards against over-exposure, levels of payment (which should be appropriate but should not be at such a level to be considered coercion), the issues of using healthy female volunteers, and the issues of supervision of the volunteers during a study.

Screening of healthy volunteers is essential, but can generate some unexpected findings.

Screening usually includes:

- Full medical history and physical examination
- Standard clinical and laboratory tests (electrocardiogram, HIV and hepatitis B screening, and screening for concealed therapeutic and recreational drugs)

A study by Singh and Williams¹ reported that between 1990-1994 of 1,293 volunteers screened 141 were rejected for medical reasons indicating that 10.9% of those screened were not healthy.

The safety of volunteers during a study is paramount. A survey by Orme et al² of medical conditions occurring in healthy volunteer studies involving the administration of medicines reported that 8,163 healthy volunteers participated in Phase 1 studies during 1986-1987. Of these 6.9% experienced minor adverse events, 0.5% experienced moderate adverse events and 0.04% experienced potentially life-threatening events.

GAFREC workshop for other attendees

Dr Richard Tiner, Medical Director of the APBI, reviewed the EU Clinical Trial Directive & Governance Arrangements For Research Ethics Committees (GAFREC) to highlight what independent Phase 1 ECs must do in order to

comply with the Directive. The Directive states that Member States must take measures necessary for the establishment and operation of Research Ethics Committees (RECs), including governance for Independent Ethics Committees. Member States have until 1st May 2003 to prepare national provisions for complying with the Directive and must adopt these provisions by 1st May 2004. GAFREC is expected to be in place by April 2003, and will cover such aspects as:

- The role of the Research Ethics Committee
- The remit of an NHS REC
- Establishment and support of NHS RECs
- Membership requirements and process
- Composition of a REC
- Working procedures
- Multi-centre research
- The process of ethical review of a research protocol
- Submitting an application

Key points included of this presentation included:

- Independent RECs will be required to show that they are free from bias
- Appointment of members shall be by an open process
- An appointed member must be prepared to publish his/her full name, profession and affiliation
- Summary of details of the REC application shall be made publicly available once the final decision is ratified by the REC
- Annual reports are to be submitted to the appointing Authority

Effect of the EU-CTD on Ethics Committees

The afternoon session commenced with a presentation from Professor Terry Stacey, Director of Central Office of Research Ethics Committees (COREC) on 'The effect of the EU Clinical Trial Directive on Ethics Committees'. A new UK Ethics Committee Authority (UKECA) will be established. UKECA will be headed by the Ministers of Health for England, Scotland, Wales and Northern Ireland. The management structures for each country will differ, but UKECA will be the overall authority. UKECA will recognise MRECs, LRECs, universities, and independent ethics committees, all of whom will have to be accredited by UKECA in order to carry out the function and responsibilities of an Ethics Committee.

Accreditation will ensure that the standards and processes of ECs are of the highest quality for ethical review, and the requirements of The Directive will ensure homogeneity throughout Europe. This accreditation will be in place by February 2004, and all existing ECs will be accredited by this date. As part of the

accreditation procedure, ECs must have a written constitution and SOPs, be able to demonstrate quality of ethical review, and have in place a training program for members.

It is currently proposed that ECs will be comprised of 18 members, one of whom will be the Chairman. A third of the membership will be 'lay members'. Members will not be allowed to serve for more than five years, and must be able to demonstrate that they have no financial interest in the business of the committee. It is anticipated that deputies will be allowed, and others can be co-opted on as members provided it can be demonstrated that they are appropriately trained. There will be an open process for recruitment of new members.

It is proposed that a quorum will be at least seven members, which must include the Chair, Vice Chair and a lay member. This group will review the primary protocol, protocol amendments and make the decision to grant ethical approval, refer or reject. It is anticipated that a subcommittee will consist of three members rather than two. A meeting can be held by telephone, providing that the meeting is scheduled and minuted.

RECs will need to produce Annual Reports for which guidance will be available. Discrete office facilities and administration together with appropriate finances and support for RECs will be a legal requirement.

The revised Green Book

Dr Michael Goggin gave a comprehensive review of the 'Green Book' – the second edition of the introduction to the work of RECs. This provides a guide to being a member on an REC considering human pharmacology (Phase 1) studies. All conference delegates were provided with a copy of the 'green book' published by the ABPI, which was released for publication that day!

Parallel workshops

Parallel workshops on "Recruitment, Inducement & Over volunteering" and "A Difficult Protocol" concluded the meeting's first day. Unfortunately, none of the authors attended the session on "A Difficult Protocol".

Dr Malcolm Boyce presented the Recruitment, Inducement & Over-Volunteering workshop. A survey of healthy volunteers recruited by Phase 1 units was presented in which a total of 6,000 healthy volunteers were involved, 68 of which were thought to have over-volunteered. Interestingly, 58 were men and only 10 were women; this amounts to a ratio of 1:100. A

demonstration of the proposed TOPS program³ was provided. TOPS stands for The Over-volunteering Prevention System, which will be used by eight UK Phase 1 Units. The purpose of this program is to identify 'over-volunteers' and has been designed to be quick and easy to use via the Internet. The Association of Independent Clinical Research Contractors⁴ (AICRC) will take over hosting the register. The delegates also took part in a volunteer payment exercise.

The Impact of the EU Directive on Phase 1

The second day of the seminar focussed on the Impact of the EU Directive on Phase 1 clinical trials, and was relevant for anybody working in this field. Delegates included those from industry, Phase 1 units and academia. The morning was devoted to an update from the Medicines Controls Agency (MCA) and the perspective on all of this from an Investigator.

MCA perspective

Dr. Elaine Godfrey, Pharmaceutical Assessor with the Medicines Controls Agency (MCA), gave an overview of the Directive 2001/20/EC (CTD) and issues relating to Phase 1 studies. Detailed below are significant issues covered by Dr Godfrey.

The definition of a clinical trial is "any investigation in human subjects..." meaning that healthy volunteer studies will be subject to the same regulatory review as patient studies. This is obviously a significant change in the UK, where volunteer studies were not previously regulated. The MCA recognises this, including the significant increase in their workload that will result. They are committed to ensuring that the UK still remains a competitive place to perform Phase 1 studies and as such have addressed some key issues:

- The MCA has committed to assessing Phase 1 study applications in a mean time of 14 and a maximum of 21 days as opposed to the 60-day timeframe referenced in the CTD – this was received with enthusiasm.
- The European Commission Guidance Note on submissions to the Competent Authority will be expanded to include a section specifically on Phase 1 studies; this was also welcomed by attendees. A draft copy of this document will be circulated to other Competent Authorities for review. It was not clear whether wider review would be sought.
- The MCA are compiling training packages for both Phase 1 and non-commercial studies. As these are still in the planning stages, they stated that they would welcome external input.

A question was raised regarding the definition of a Phase 1 study by the MCA - Dr Godfrey

confirmed that current thinking included only healthy volunteer studies and special patient populations (e.g. studies in renally and hepatically impaired patients). However, it was raised by delegates that there are examples of Phase 1 studies in patients designed to show a physiological effect, which could not be of therapeutic benefit to those patients involved, and that these have not previously required approval. Dr Godfrey asked that details of such studies be provided via the ABPI for the MCA to consider further...

Dr Godfrey told delegates that the MCA Clinical Trials Unit is planning to implement a Phase 1 pilot. This is likely to take place in mid-2003, and it will cost £600 for pharmaceutical companies to volunteer to take part. (More details of this are available on AHPPI website⁵).

Next, Dr Godfrey noted that the definition of an Investigational Medicinal Product (IMP) is any active substance or placebo, and therefore includes comparators and placebo; as such, GMP will apply them too. Handling of IMPs will be reviewed by GCP auditors as part of an audit of a Phase 1 facility.

Another significant issue included in her presentation was the 'loss of the DDX', which will have significant implications for a number of people represented at the meeting as there is no longer a different process for investigator initiated studies.

Finally, picking up on the theme of the first day, Dr Godfrey reminded us again that the Directive provides a statutory basis for Ethics committees and GCP, once transcribed into UK law. This will also apply to independent IECs linked to Phase 1 units. The Directive also includes requirements for safety reporting.

Mr Ian Oulsnam, MCA GCP inspector went on to give an overview of the issues relating to inspection of Phase 1 clinical units and findings from recent audits. Mr Oulsnam also pointed out that the Directive introduces new powers in the UK:

- Power to inspect any site involved with a clinical trial in the UK.
- Power to levy fees for the conduct of an inspection - MCA currently charge £5000.

An overview of a typical GCP inspection of a Phase 1 unit was provided. He stated that the 'independence' of ethics committees would be assessed for units who use independent committees. Additionally, IMP handling and validation, etc. of all bespoke computer systems would be included in an audit

The main findings were then presented from the MCA voluntary inspections that have been performed to date. These include:

- Volunteer medical history/eligibility information not received from GPs
- Significant protocol amendments implemented prior to EC approval or following Chairman's action
- Inadequate procedures for nursing/physician cover out of normal working hours
- Inadequate procedures/facilities for the preparation of IMPs
- Inadequate procedures for unblinding blinded subjects in an emergency
- Inadequacies relating to contracts/agreements with sponsors, agencies and contractors
- Lack of recall procedures for emergency medication, support medication and comparator drugs
- Inadequate training of agency staff

Finally, Mr Oulsnam told delegates that consultation is underway prior to introduction of the new UK statutory instrument relating to mandatory inspections. A number of people have been asked to be involved in the process and the Institute will be asked to provide representation on behalf of our members.

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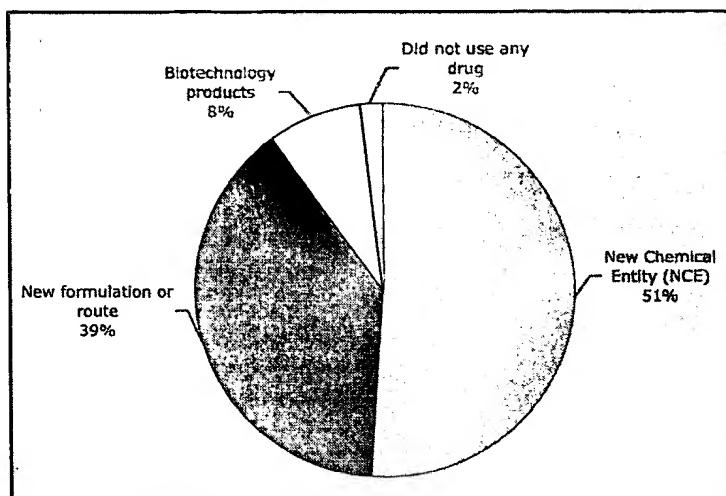
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The Investigator perspective

Dr. Steve Warrington's presentation considered the impact of the Directive from the viewpoint of an investigator at a Phase 1 unit. In order to give the audience an idea of the studies performed in a unit, he presented an analysis of over 300 studies performed at his unit between 1993 and 2001, and described the types of studies performed (see below):



Dr Warrington's impression was that a significant impact of the CTD would be the time taken to gain approval from the Competent Authority. He was reassured by the MCA's commitment to a 14-day review period for the original submission; however, he highlighted that an area of particular concern was the approval of protocol amendments. This relates to the requirement to submit all 'substantial amendments' to the Competent Authority prior to their implementation (Article 10a). A number of points were made around the definition of 'substantial', as this is not clearly defined in the Article.

In order to describe his concerns, he presented data on the number and type of amendments produced for 280 studies. 143 amendments were given expedited review by the REC and these regularly included additional measurements, etc. (see opposite):

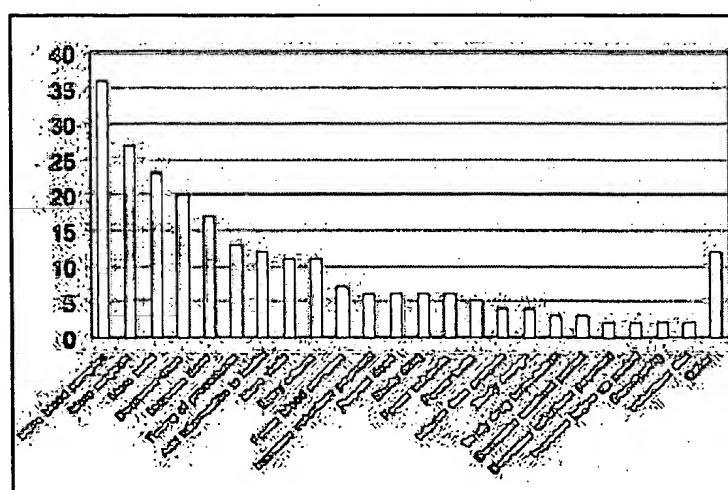
Under the current definition, many of these amendments would be considered 'substantial'. These amendments are often produced in Phase 1 studies based on emerging data and usually need to be implemented for the next dose in a study, which can be as little as 7 days later. The mean approval time for these amendments by Dr Warrington's local REC was 4 days and this is representative of the majority of Phase 1 units. This rapid review and approval is essential to ensure that the most appropriate data is obtained; it could be argued that it would be unethical to

continue as before if the data collected is not the providing the most informed data to guide the early development programme. He suggested that in order to avoid this time-consuming step, sponsors may consider producing all-embracing protocols and he provided an example. In addition, he also provided examples of 'ultra-flexible protocols' for example a protocol might state 'extra assessments of BP, pulse rate and ECGs may be included depending on emerging data' or 'a dose range between Xmg and Ymg will be explored', rather than documenting actual doses to be studied.

Dr Warrington concluded by presenting some data from an AHPP survey showing that >600 studies and >100 protocol amendments per year will be submitted for review. The majority of amendments will require expedited review and he stated that if Phase 1 units in the UK are to remain competitive, the response time from the MCA must match the current ethics committee timelines. He also stated that a definition of 'substantial amendments' for submission to the MCA is urgently needed.

Academic research

Dr Sally Burtles, Director of Drug Development for Cancer Research UK provided a very interesting presentation on the perceived impact of the CTD on academic and investigator-led studies. Cancer Research UK is the largest UK charity and provides the major funding of cancer research in the UK. They have an extensive trials portfolio focussing on early stage development of novel treatments and Phase 3 trials of licensed products. Therefore, Dr Burtles was well placed to provide this overview.



Dr Burtles highlighted three major areas of the Directive that will impact on academic clinical research:

- All clinical trials have to be undertaken to GCP standards

- All clinical trials supplies have to be made to Good Manufacturing Practice (GMP) standards in a licensed facility
- The loss of the DDX

She stated that, whilst the academic community can comply with all of these requirements in principle, the major barriers are costs (which will increase significantly) and the lack of relevant expertise within the academic community.

The requirement for GCP compliance is a major issue. Although Cancer Research UK's early clinical trials are undertaken to ICH GCP standards, the vast majority of academic clinical trials are not. Many studies do not have a single defined sponsor, with multiple individuals and agencies taking on different responsibilities. Most clinical groups can operate to GCP, as they do so when they undertake trials with companies; however, they probably do not have the documentation, systems or staff to operate to GCP themselves. GCP covers all aspects of a clinical trial, including the laboratory activities (PK and PD) and functional imaging assessments. The majority of academic labs do not have any quality systems in place, and a great deal of work and commitment will be needed in order to achieve acceptable standards by May 2004.

Dr Burtles also highlighted that getting novel agents developed to enable them to be made to GMP will increase the cost by 3-4x over the 'GMP-like' standards that are currently utilised by Cancer Research UK. For other academics who currently manufacture their own supplies (e.g. antibodies), they will no longer be able to do this and to contract production out will increase costs by an order of magnitude. Costs to contract out production are already very high, but there are insufficient licensed production facilities, which will allow the costs to increase further as demand outstrips supply. In addition to the cost, the academic community generally lacks the expertise required for understanding the issues around GMP production and release of materials for human use. A further related issue is the need for a Qualified Person (QP) to release the supplies for use, as the majority of academic investigator sites do not have this expertise available.

It is expected that the loss of the DDX will have a major impact on academic clinical research, particularly in the development of novel agents to be tested in Phase 1 and 2 trials. The proposed application procedure will require an extensive application with supporting data. There are concerns around the application that include having the expertise to write the dossier, and the



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potential increase in costs to undertake more extensive pre-clinical studies.

Dr Burtles estimated that the cost of taking a new agent from academic research into Phase 1 clinical trials could increase by about 3 to 4 fold under the requirements of the Directive and GCP. She concluded that the Directive will have a major impact on all of us and we need to minimise the negative impact on academic clinical trials so that we can continue to provide data that will help improve the treatments available to cancer patients worldwide.

GMP/IMP issues

Mr Alan Newbery went over several Articles of the Directive, but concentrated on the issue of GMP requirements for IMP, including the necessary GMP procedures investigational sites will need to have in place.

He quoted some background information: the Directive refers to Directive 65/65/EEC and 75/318/EEC and the Declaration of Helsinki. He stated that 19 paragraphs and 23 articles cover everything from payments to minors (Art. 4) through to changes to scientific progress to GMP requirements (Art. 13 & 15). There is a clear definition for a clinical trial and also an IMP. An

IMP is also defined in Article 2. Annex 13 of GMP, which is still a draft, covers the manufacture of investigational medicinal products. It covers issues such as quality management, personnel, premises and equipment, documentation requirements, manufacturing processes and their validation and packaging and labelling.

Under the new Directive the release of IMP for use in a clinical trial must be performed by a Qualified Person (QP), a European qualification only. The QP must ensure that the IMP has been manufactured in accordance with the requirements of European GMP. If an IMP is sourced from outside the EU the QP must also ensure the IMP has been manufactured in accordance with European GMP and the EU-CTD. Sourcing of IMPs outside of the EU may present a number of problems for QP release in order to satisfy the requirements of the Directive.

Pharmaceutical Industry Competitiveness Task Force

Dr. Richard Tiner gave the last presentation of the day on the Pharmaceutical Industry Competitiveness Task Force (PICTF). PICTF was established during a meeting at No.10 between the Prime Minister, Sir Richard Sykes and Sir Tom McKillop. Dr Tiner said that the UK is an expensive

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place to do research but the quality of this research is good. He stated that 40% of clinical research is conducted by sponsor companies. At the initial meeting, the remit of PICTF was agreed and an action plan devised. This included:

1. Improve start up times from April 2001
2. DOH Research Governance Framework
3. Partnership agreement
4. Transparency in costing
5. Agreed performance indicators

The PICTF Clinical Research Group highlighted a need for a partnership between Industry and the NHS. The Research Partnership Agreement is PICTF's first completed action. A copy of this is available from the ABPI and Department of Health websites. The ABPI policy on registration of clinical trials has been prepared and should go live shortly. PICTF have worked with the MCA to ensure that the review timeframe for a CTX does not increase and this has been achieved; additionally they are working together to ensure that the percentage of CTX's granted within 36 days does not fall.

Costs and overheads have been investigated and there is increasing transparency within Trusts. It appears that some companies have historically been charged for participants' standard care during a clinical trial, whereas only treatment outside standard care should be charged for. Dr Tiner said that some NHS/Universities had been 'double-costing', and that the MRC were also unsure of what the costs were for.

To save time and money in setting up studies, a Model Clinical Trials Agreement has been written and is currently being reviewed by lawyers. The Agreement is a template for a contract between an NHS Trust and a sponsor. It is hoped that Sponsor companies and NHS Trusts will use this to ensure a timely and prompt start up of clinical trials in England. This should also promote consistency around the costs of a clinical trial.

Ongoing activities include the COREC novel processes review and the Good Publication Policy (available in Spring 2003). Data protection issues are also under review, as 15% of international studies have UK patients. Feasibility studies are currently being conducted to understand the issues. Clinical Research Networks are also being encouraged, with the Cancer Research Network as a good example already in existence. Other networks to be set up include mental health and paediatrics.

A benchmarking exercise is underway with 20 companies providing data. This exercise will be

used to understand whether the implementation of the recommendations is ensuring that the UK remains at the forefront of clinical research. Early results show that the time for REC review is decreasing. However, 10-20% of REC applications are not valid as the application is not complete when submitted.

Dr Tiner was also asked to provide insight on the status of the implementation of the Directive outside the UK. He informed the participants that the situation outside the UK is not clear. However, current information suggests that the process in The Netherlands will not change significantly and the role of the Competent Authority will be performed by the CCMO (a central 'ethics committee') for most products. Currently the CCMO only deals with 'special products' such as gene therapy. The submission to Competent Authority is likely to take the form of a 'mini Common Technical Document' but this has not yet been finalised. There are some perceived difficulties in Germany, but the Scandinavian countries are moving forward in an efficient manner. However, the status in the Eastern Block is unknown. He concluded by stating that there isn't much information available on the implementation of the Directive in other countries and EFPIA are trying to build a database.

A short summary of the implications for RECs, as covered on the first day of this 2-day seminar, was the closing item of a very useful and interesting seminar, the first organised by the ABPI and the Institute of Clinical Research Phase 1-Sub-Committee.

Liz Allen is Director of Scientific Affairs, GDRU Quintiles Ltd, and Vice-Chair of the Phase 1 Sub-Committee, Annalies Legters is International Clinical Trial Coordinator, Lundbeck BV, Angie Major is Membership Development Manager, Institute of Clinical Research, Lesley Shelford is Senior CRA, Janssen-Cilag Ltd, and Lisa Tan is Clinical Project Scientist, Pfizer and Chair of the Phase 1 Sub-Committee, Annalies and Lesley are also members of the Phase 1 Sub-Committee.

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London, 26 July 2001
CPMP/EWP/QWP/1401/98

**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS
(CPMP)**

**NOTE FOR GUIDANCE ON
THE INVESTIGATION OF BIOAVAILABILITY AND BIOEQUIVALENCE**

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Note:

This revised Note for Guidance will replace the previous guideline adopted in December 1991.

**NOTE FOR GUIDANCE ON INVESTIGATION OF BIOAVAILABILITY
AND BIOEQUIVALENCE**

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1 INTRODUCTION

To exert an optimal therapeutic action an active moiety should be delivered to its site of action in an effective concentration for the desired period. To allow reliable prediction of the therapeutic effect the performance of the dosage form containing the active substance should be well characterised.

In the past, several therapeutic misadventures related to differences in bioavailability (e.g. digoxin, phenytoin, primidone) testify to the necessity of testing the performance of dosage forms in delivering the active substance to the systemic circulation and thereby to the site of action. Thus the bioavailability of an active substance from a pharmaceutical product should be known and reproducible. This is especially the case if one product containing one certain active substance is to be used instead of its innovator product. In that case the product should show the same therapeutic effect in the clinical situation. It is generally cumbersome to assess this by clinical studies.

Comparison of therapeutic performances of two medicinal products containing the same active substance is a critical means of assessing the possibility of alternative use between the innovator and any essentially similar medicinal product. Assuming that in the same subject an essentially similar plasma concentration time course will result in essentially similar concentrations at the site of action and thus in an essentially similar effect, pharmacokinetic data instead of therapeutic results may be used to establish equivalence: bioequivalence.

It is the objective of this guidance to define, for products with a systemic effect, when bioavailability or bioequivalence studies are necessary and to formulate requirements for their design, conduct, and evaluation. The possibility of using *in vitro* instead of *in vivo* studies with pharmacokinetic end points is also envisaged.

This guideline should be read in conjunction with Directive 75-318/EEC, as amended, and other pertinent elements outlined in current and future EU and ICH guidelines and regulations especially those on:

- Pharmacokinetic Studies in Man
- Modified Release Oral and Transdermal Dosage Forms: Section I (Pharmacokinetic and Clinical Evaluation)
- Modified Release Oral and Transdermal Dosage Forms: Section II (Quality)
- Investigation of Chiral Active Substances.
- Fixed Combination Medicinal Products
- Clinical Requirements for Locally Applied, Locally Acting Products Containing Known Constituents.
- The Investigation of Drug Interactions
- Development Pharmaceutics
- Process Validation
- Manufacture of the Finished Dosage Form
- Validation of analytical procedures: Definitions and Terminology (ICH topic Q2A)
- Validation of analytical procedures: Methodology (ICH topic Q2B)
- Structure and Content of Clinical Study Reports (ICH topic E3)
- Good Clinical Practice: Consolidated Guideline (ICH topic E6)
- General Considerations for Clinical Trials (ICH topic E8)
- Statistical Principles for Clinical Trials (ICH topic E9)
- Choice of Control Group in Clinical Trials (ICH topic E10)
- Amendments to Commission Regulation on (EC) 542/95
- Common Technical Document (ICH topic M4)

For medicinal products not intended to be delivered into the general circulation the common

systemic bioavailability approach cannot be applied. Under these conditions the (local) availability may be assessed, where necessary, by measurements quantitatively reflecting the presence of the active substance at the site of action using methods specially chosen for that combination of active substance and localisation (see section 5.1.8). In this case, as well as in others, alternative methods may be required such as studies using pharmacodynamic end points. Furthermore, where specific requirements for different types of products are needed, the appropriate exceptions are mentioned therein.

This Note for Guidance does not explicitly apply to biological products.

2 DEFINITIONS

Before defining bioavailability and related terminology some definitions pertaining to dosage and chemical forms are given:

2.1 Pharmaceutical equivalence

Medicinal products are pharmaceutically equivalent if they contain the same amount of the same active substance(s) in the same dosage forms that meet the same or comparable standards.

Pharmaceutical equivalence does not necessarily imply bioequivalence as differences in the excipients and/or the manufacturing process can lead to faster or slower dissolution and/or absorption.

2.2 Pharmaceutical alternatives

Medicinal products are pharmaceutical alternatives if they contain the same active moiety but differ in chemical form (salt, ester, etc.) of that moiety or in the dosage form or strength.

2.3 Bioavailability

Bioavailability means the rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action.

In the majority of cases substances are intended to exhibit a systemic therapeutic effect, and a more practical definition can then be given, taking into consideration that the substance in the general circulation is in exchange with the substance at the site of action:

-Bioavailability is understood to be the extent and the rate at which a substance or its active moiety is delivered from a pharmaceutical form and becomes available in the general circulation.

It may be useful to distinguish between the "absolute bioavailability" of a given dosage form as compared with that (100%) following intravenous administration (e.g. oral solution *vs.* iv.), and the "relative bioavailability" as compared with another form administered by the same or another non intravenous route (e.g. tablets *vs.* oral solution).

2.4 Bioequivalence

Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.

Alternatively to classical bioavailability studies using pharmacokinetic end points to assess bioequivalence, other types of studies can be envisaged, e.g. human studies with clinical or pharmacodynamic end points, studies using animal models or in vitro studies as long as they are appropriately justified and/or validated.

2.5 Essentially similar products

The current EU definition for essentially similar products is as follows (see "The rules governing medicinal products in the European Union", Notice to Applicants, Vol. 2A in accordance with the December 1998 European Court of Justice ruling in the "Generics" case):

"A medicinal product is essentially similar to an original product where it satisfies the criteria of having the same qualitative and quantitative composition in terms of active substances, of having the same pharmaceutical form, and of being bioequivalent unless it is apparent in the light of scientific knowledge that it differs from the original product as regards safety and efficacy".

By extension, it is generally considered that for immediate release products the concept of essential similarity also applies to different oral forms (tablets and capsules) with the same active substance.

The need for a comparative bioavailability study to demonstrate bioequivalence is identified under 5.1. Concerns about differences in essentially similar medicinal products lie on the use of different excipients and methods of manufacture that ultimately might have an influence on safety and efficacy. A bioequivalence study is the widely accepted means of demonstrating that these differences have no impact on the performance of the formulation with respect to rate and extent of absorption, in the case of immediate release dosage forms. It is desirable that excipients must be devoid of any effect or their safe use is ensured by appropriate warning in the package label – see guideline on excipients in the label and package leaflet: "The Rules Governing Medicinal Products in the European Union", 1998, Vol. 3B, - and not interfere with either the release or the absorption process.

An essentially similar product can be used instead of its innovator product. An 'innovator' product is a medicinal product authorised and marketed on the basis of a full dossier i.e. including chemical, biological, pharmaceutical, pharmacological-toxicological and clinical data. A 'Reference Product' must be an 'innovator' product (see 3.5).

2.6 Therapeutic equivalence

A medicinal product is therapeutically equivalent with another product if it contains the same active substance or therapeutic moiety and, clinically, shows the same efficacy and safety as that product, whose efficacy and safety has been established.

In practice, demonstration of bioequivalence is generally the most appropriate method of substantiating therapeutic equivalence between medicinal products, which are pharmaceutically equivalent or pharmaceutical alternatives, provided they contain excipients generally recognised as not having an influence on safety and efficacy and comply with labelling requirements with respect to excipients. (see 2.5).

However, in some cases where similar extent of absorption but different rates of absorption are observed the products can still be judged therapeutically equivalent if those differences are not of therapeutic relevance. A clinical study to prove that differences in absorption rate are not therapeutically relevant will probably be necessary.

3 DESIGN AND CONDUCT OF STUDIES

In the following sections, requirements for the design and conduct of bioavailability or bioequivalence studies are formulated. It is assumed that the applicant is familiar with pharmacokinetic theories underlying bioavailability studies. The design should be based on a reasonable knowledge of the pharmacodynamics and/or the pharmacokinetics of the active substance in question. For the pharmacokinetic basis of these studies reference is made to the recommendation "Pharmacokinetic studies in man". The design and conduct of the study

should follow EU-regulations on Good Clinical Practice, including reference to an Ethics Committee.

A bioequivalence study is basically a comparative bioavailability study designed to establish equivalence between test and reference products. The following sections apply mainly to bioequivalence studies. Since bioavailability studies are comparative in nature, the contents of the following sections apply to these studies as well, with the necessary adaptations in accordance with the aim of each specific study. Where necessary, specific guidance concerning bioavailability studies will be given.

The methodology of bioequivalence studies can be used to assess differences in the pharmacokinetic parameters in pharmacokinetic studies such as drug-drug or food-drug interactions or to assess differences in subsets of the population. In this case the relevant guidelines should be followed and the selection of subjects, the design and the statistical analysis should be adjusted accordingly.

3.1 Design

The study should be designed in such a way that the formulation effect can be distinguished from other effects. If the number of formulations to be compared is two, a two-period, two-sequence crossover design is often considered to be the design of choice.

However, under certain circumstances and provided the study design and the statistical analyses are scientifically sound alternative well-established designs could be considered such as parallel design for very long half-life substances and replicate designs for substances with highly variable disposition.

In general, single dose studies will suffice, but there are situations in which steady-state studies

- may be required, e.g. in the case of
 - dose- or time-dependent pharmacokinetics,
 - some modified release products (in addition to single dose investigations),
- or can be considered, e.g.
 - if problems of sensitivity preclude sufficiently precise plasma concentration measurements after single dose administration.
 - if the intra-individual variability in the plasma concentration or disposition precludes the possibility of demonstrating bioequivalence in a reasonably sized single dose study and this variability is reduced at steady state.

In such steady-state studies the administration scheme should follow the usual dosage recommendations.

The number of subjects required is determined by

- a) the error variance associated with the primary characteristic to be studied as estimated from a pilot experiment, from previous studies or from published data,
- b) the significance level desired,
- c) the expected deviation from the reference product compatible with bioequivalence (δ) and
- d) the required power.

The clinical and analytical standards imposed may also influence the statistically determined number of subjects. However, generally the minimum number of subjects should be not smaller than 12 unless justified.

Subsequent treatments should be separated by adequate wash out periods. In steady-state studies wash out of the previous treatment last dose can overlap with the build-up of the second treatment, provided the build-up period is sufficiently long (at least three times the terminal half-life).

The sampling schedule should be planned to provide an adequate estimation of C_{max} and to cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of absorption. This is generally achieved if the AUC derived from measurements is at least 80% of the AUC extrapolated to infinity. If a reliable estimate of terminal half-life is necessary, it should be obtained by collecting at least three to four samples during the terminal log linear phase.

In order to study bioavailability under steady-state conditions when differences between morning and evening or nightly dosing are known, (e.g. if it is known that the circadian rhythm is known to have an influence on bioavailability), sampling should be carried out over a full 24 hours cycle.

For drugs with a long half-life, relative bioavailability can be adequately estimated using truncated AUC as long as the total collection period is justified. In this case the sample collection time should be adequate to ensure comparison of the absorption process.

3.2 Subjects

3.2.1 Selection of subjects

The subject population for bioequivalence studies should be selected with the aim to minimise variability and permit detection of differences between pharmaceutical products. Therefore, the studies should normally be performed with healthy volunteers. The inclusion/exclusion criteria should be clearly stated in the protocol.

Subjects could belong to either sex; however, the risk to women of childbearing potential should be considered on an individual basis.

In general, subjects should be between 18 - 55 years old and of weight within the normal range according to accepted normal values for the Body Mass Index. They should be screened for suitability by means of clinical laboratory tests, an extensive review of medical history, and a comprehensive medical examination. Depending on the drug's therapeutic class and safety profile special medical investigations may have to be carried out before, during and after the completion of the study. Subjects should preferably be non-smokers and without a history of alcohol or drug abuse. If moderate smokers are included (less than 10 cigarettes per day) they should be identified as such and the consequences for the study results should be discussed.

3.2.2 Standardisation of the study

The test conditions should be standardised in order to minimise the variability of all factors involved except that of the products being tested. Therefore, standardisation of the diet, fluid intake and exercise is recommended. Subjects should preferably be fasting at least during the night prior to administration of the products. If the Summary of Product Characteristics of the reference product contains specific recommendations in relation with food intake related to food interaction effects the study should be designed accordingly.

The time of day for ingestion should be specified and as fluid intake may profoundly influence gastric passage for oral administration forms, the volume of fluid (at least 150 ml) should be constant. All meals and fluids taken after the treatment should also be standardised in regard to composition and time of administration during the sampling period. The subjects should not take other medicines during a suitable period before and during the study and should abstain from food and drinks, which may interact with circulatory, gastrointestinal,

liver or renal function (e.g. alcoholic or xanthine-containing beverages or certain fruit juices). As the bioavailability of an active moiety from a dosage form could be dependent upon gastrointestinal transit times and regional blood flows, posture and physical activity may need to be standardised.

3.2.3 Inclusion of patients

If the investigated active substance is known to have adverse effects and the pharmacological effects or risks are considered unacceptable for healthy volunteers it may be necessary to use patients instead, under suitable precautions and supervision. In this case the applicant should justify the alternative.

3.2.4 Genetic phenotyping

Phenotyping and/or genotyping of subjects should be considered for exploratory bioavailability studies and all studies using parallel group design. It may be considered as well in crossover studies (e.g. bioequivalence, dose proportionality, food interaction studies etc.) for safety or pharmacokinetic reasons. If a drug is known to be subject to major genetic polymorphism, studies could be performed in panels of subjects of known phenotype or genotype for the polymorphism in question.

3.3 Characteristics to be investigated

In most cases evaluation of bioavailability and bioequivalence will be based upon the measured concentrations of the parent compound. In some situations, however, measurements of an active or inactive metabolite may be necessary instead of the parent compound. Such situations include cases where the use of a metabolite may be advantageous to determine the extent of drug input, e.g. if the concentration of the active substance is too low to be accurately measured in the biological matrix (e.g. major difficulty in analytical method, product unstable in the biological matrix or half-life of the parent compound too short) thus giving rise to significant variability.

Bioequivalence determinations based on metabolites should be justified in each case bearing in mind that the aim of a bioequivalence study is intended to compare the *in vivo* performance of test and reference products. In particular if metabolites significantly contribute to the net activity of an active substance and the pharmacokinetic system is non-linear, it is necessary to measure both parent drug and active metabolite plasma concentrations and evaluate them separately.

In bioavailability studies, the shape of and the area under the plasma concentration *versus* time curves are mostly used to assess extent and rate of absorption. The use of urine excretion data may be advantageous in determining the extent of drug input in case of products predominately excreted renally, but has to be justified when used to estimate the rate of absorption. Sampling points or periods should be chosen, such that the time- concentration profile is adequately defined so as to allow the estimation of relevant parameters.

From the primary results, the bioavailability characteristics desired are estimated, namely AUC_t , AUC_{∞} , C_{\max} , t_{\max} , Ae_t , Ae_{∞} as appropriate, or any other justifiable characteristics (cf. Appendix I). The method of estimating AUC -values should be specified. For additional information $t_{1/2}$ and MRT can be estimated. For studies in steady state AUC_{τ} , C_{\max} , C_{\min} and fluctuation should be provided.

In bioequivalence studies the AUC is the most reliable reflection of the extent of absorption.

The exclusive use of compartmental based estimates are not recommended.

If pharmacodynamic effects are used as characteristics the measurements should provide a sufficiently detailed time course, the initial values in each period should be comparable and the complete effect curve should remain below the maximum physiological response.

Specificity, accuracy and reproducibility of the methods should be sufficient. The non-linear character of the dose/response relationship should be taken into account and base line corrections should be considered during data analysis.

3.4 Chemical analysis

The bioanalytical part of bioequivalence trials should be conducted according to the applicable principles of Good Laboratory Practice (GLP).

The bioanalytical methods used to determine the active moiety and/or its biotransformation product(s) in plasma, serum, blood or urine or any other suitable matrix must be well characterised, fully validated and documented to yield reliable results that can be satisfactorily interpreted. The main objective of method validation is to demonstrate the reliability of a particular method for the quantitative determination of an analyte(s) concentration in a specific biological matrix. The characteristics of a bioanalytical method essential to ensure the acceptability of the performance and the reliability of analytical results are: (1) stability of the stock solutions and of the analyte(s) in the biological matrix under processing conditions and during the entire period of storage; (2) specificity; (3) accuracy; (4) precision (5) limit of quantification and (6) response function.

The validation of a bioanalytical method should comprise two distinct phases: (1) the pre-study phase in which the compliance of the assay with the six characteristics listed above is verified and (2) the study phase itself in which the validated bioanalytical method is applied to the actual analysis of samples from the biostudy mainly in order to confirm the stability, accuracy and precision.

A calibration curve should be generated for each analyte in each analytical run and it should be used to calculate the concentration of the analyte in the unknown samples in the run. A number of separately prepared Quality Control samples should be analysed with processed test samples at intervals based on the total number of samples. In addition, it is necessary to validate the method of processing and handling the biological samples.

All procedures should be performed according to pre-established Standard Operating Procedures (SOPs). All relevant procedures and formulae used to validate the bioanalytical method should be submitted and discussed. Any modification of the bioanalytical method before and during analysis of study specimens may require adequate revalidation; all modifications should be reported and the scope of revalidation justified.

According to the requirements of the note for guidance on the "Investigation of Chiral Active Substances", bioequivalence studies supporting applications for essentially similar medicinal products containing chiral active substances should be based upon enantiomeric bio-analytical methods unless (1) both products contain the same stable single enantiomer; (2) both products contain the racemate and both enantiomers show linear pharmacokinetics.

3.5 Reference and test product

Test products in an application for a generic product are normally compared with the corresponding dosage form of an innovator (see 2.5) medicinal product (reference product). The choice of reference product should be justified by the applicant.

For an abridged application claiming essential similarity to a reference product, application to numerous Member States based on bioequivalence with a reference product from one Member State can be made.

Such an application can be considered acceptable unless there is a significant difference between the reference products originating from the same manufacturer (or its subsidiaries/licensees), in terms of the qualitative and quantitative composition in excipients. Concerned Member States may request information from the first Member State on the

reference product, namely on the composition, manufacturing process and finished product specification.

Where additional bioequivalence studies are required, they should be carried out using the product registered in the concerned Member State as the reference product

It should be remembered that the development of the test product should always take into account the Note for Guidance on "Development Pharmaceutics".

The test products used in the biostudy must be prepared in accordance with GMP-regulations. Batch control results of the test product should be reported.

In the case of oral solid forms for systemic action the test product should usually originate from a batch of at least 1/10 of production scale or 100 000 units, whichever is greater, unless otherwise justified. The production of batches used should provide a high level of assurance that the product and process will be feasible on an industrial scale; in case of production batch smaller than 100 000 units, a full production batch will be required. If the product is subjected to further scale-up this should be properly validated.

Samples of the product from full production batches should be compared with those of the test batch, and should show similar in vitro dissolution profiles when employing suitable dissolution test conditions (see Appendix II).

The study sponsor will have to retain a sufficient number of all investigational product samples in the study for one year in excess of the accepted shelf life or two years after completion of the trial or until approval whichever is longer to allow re-testing, if it is requested by the authorities.

In accordance with Annex 13 to the EU guide to GMP, reference and test product must be packed in an individual way for each subject included in the bioequivalence trial. Every effort should be made to allow a precise tracking of administration of the reference and test products to the subjects, for instance by the use of labels with a tear-off portion.

3.6 Data analysis

The primary concern of bioequivalence assessment is to quantify the difference in bioavailability between the reference and test products and to demonstrate that any clinically important difference is unlikely.

3.6.1 Statistical analysis

The statistical method for testing relative bioavailability (e.g. bioequivalence) is based upon the 90% confidence interval for the ratio of the population means (Test/Reference), for the parameters under consideration.

This method is equivalent to the corresponding two one-sided test procedure with the null hypothesis of bioinequivalence at the 5% significance level. The statistical analysis (e.g. ANOVA) should take into account sources of variation that can be reasonably assumed to have an effect on the response variable. A statistically significant sequence effect should be handled appropriately.

Pharmacokinetic parameters derived from measures of concentration, e.g. AUC, C_{max} should be analysed using ANOVA. The data should be transformed prior to analysis using a logarithmic transformation.

If appropriate to the evaluation the analysis technique for t_{max} should be non-parametric and should be applied to untransformed data. For all pharmacokinetic parameters of interest in addition to the appropriate 90% confidence intervals for the comparison of the two formulations, summary statistics such as median, minimum and maximum should be given.

3.6.2 Acceptance range for pharmacokinetic parameters

The pharmacokinetic parameters to be tested, the procedure for testing and the acceptance ranges should be stated beforehand in the protocol.

In studies to determine average bioequivalence the acceptance intervals for the main characteristics are detailed as follows:

AUC-ratio

The 90% confidence interval for this measure of relative bioavailability should lie within an acceptance interval of 0.80-1.25. In specific cases of a narrow therapeutic range the acceptance interval may need to be tightened.

In rare cases a wider acceptance range may be acceptable if it is based on sound clinical justification.

C_{max}-ratio

The 90% confidence interval for this measure of relative bioavailability should lie within an acceptance interval of 0.80-1.25. In specific cases of a narrow therapeutic range the acceptance interval may need to be tightened.

In certain cases a wider interval may be acceptable. The interval must be prospectively defined e.g. 0.75-1.33 and justified addressing in particular any safety or efficacy concerns for patients switched between formulations.

Others

Statistical evaluation of t_{max} only makes sense if there is a clinically relevant claim for rapid release or action or signs related to adverse effects. The non-parametric 90% confidence interval for this measure of relative bioavailability should lie within a clinically determined range.

For other (see 3.3) pharmacokinetic parameters in comparison relative bioavailability (e.g. C_{min}, Fluctuation, t_{1/2}, etc.) considerations analogous to those for AUC, C_{max} or t_{max} apply, taking into consideration the use of log-transformed or untransformed data, respectively.

3.6.3 Handling deviations from the study plan

The method of analysis should be planned in the protocol. The protocol should also specify methods for handling drop-outs and for identifying biologically implausible outliers. Post hoc exclusion of outliers is generally not accepted. If modelling assumptions made in the protocol (e.g. for extrapolating AUC to infinity) turn out to be invalid, a revised analysis in addition to the planned analysis (if this is feasible) should be presented and discussed.

3.6.4 A remark on individual and population bioequivalence

To date, most bioequivalence studies are designed to evaluate average bioequivalence. Experience with population and individual bioequivalence studies is limited. Therefore, no specific recommendation is given on this matter.

3.7 In vitro dissolution complementary to a bioequivalence study

The results of "in vitro" dissolution tests, obtained with the batches of test and reference products that were used in the bioequivalence study should be reported. The results should be reported as profiles of percent of labelled amount dissolved versus time.

The specifications for the *in vitro* dissolution of the product should be derived from the dissolution profile of the batch that was found to be bioequivalent to the reference product and would be expected to be similar to those of the reference product (see Appendix II).

For immediate release products, if the dissolution profile of the test product is dissimilar

compared to that of the reference product and the in vivo data remain acceptable the dissolution test method should be re-evaluated and optimised. In case that no discriminatory test method can be developed which reflects in vivo bioequivalence a different dissolution specification for the test product could be set.

3.8 Reporting of results

The report of a bioavailability or a bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with GCP-rules and related EU and ICH E3 guidelines. This implies that the authenticity of the whole of the report is attested by the signature of the principal investigator. The responsible investigator(s), if any, should sign for their respective sections of the report.

Names and affiliations of the responsible investigator(s), site of the study and period of its execution should be stated. The names and batch numbers of the products used in the study as well as the composition(s), finished product specifications and comparative dissolution profiles should be provided. In addition, the applicant should submit a signed statement confirming that the test product is the same as the one that is submitted for marketing authorisation.

All results should be clearly presented and should include data from subjects who eventually dropped-out. Drop-out and withdrawal of subjects should be fully documented and accounted for. The method used to derive the pharmacokinetic parameters from the raw data should be specified. The data used to estimate AUC should be reported. If pharmacokinetic models are used to evaluate the parameters the model and computing procedure used should be justified. Deletion of data should be justified.

All individual subject data should be given and individual plasma concentration/time curves presented in linear/linear and log/linear scale. The analytical report should include the results for all standard and quality control samples as well. A representative number of chromatograms or other raw data should be included covering the whole concentration range for all, standard and quality control samples as well as the specimens analysed. The analytical validation report should be submitted as well.

The statistical report should be sufficiently detailed to enable the statistical analysis to be repeated, e.g. randomisation scheme, demographic data, values of pharmacokinetic parameters for each subject, descriptive statistics for each formulation and period. A detailed ANOVA and/or non-parametric analysis, the point estimates and corresponding confidence intervals including the method of their estimation should also be included.

4 APPLICATIONS FOR PRODUCTS CONTAINING NEW ACTIVE SUBSTANCES

4.1 Bioavailability

In the case of new active substances (new chemical entities) intended for systemic action, the pharmacokinetic characterisation will have to include the determination of the systemic availability of the substance in its intended pharmaceutical form in comparison with intravenous administration. If this is not possible (e.g. not technically feasible or for safety reasons) the bioavailability relative to a suitable oral solution or suspension should be determined. In the case of a prodrug the intravenous reference solution should preferably be made of the active moiety.

4.2 Bioequivalence

During development bioequivalence studies are necessary as bridging studies between (i) pivotal and early clinical trial formulations; (ii) pivotal clinical trial formulations, especially those used in the dose finding studies, and the to-be-marketed medicinal product; (iii) other

comparisons depending on the situation. Such studies may be exempted if the absence of differences in the in vivo performance can be justified by satisfactory in vitro data (see 5.1.1 and 5.2).

5 APPLICATIONS FOR PRODUCTS CONTAINING APPROVED ACTIVE SUBSTANCES

5.1 Bioequivalence studies

In vivo bioequivalence studies are needed when there is a risk that possible differences in bioavailability may result in therapeutic inequivalence.

The kind of studies to be performed may vary with the type of product, as follows.

5.1.1 Oral Immediate Release Forms with Systemic Action

This section pertains to dosage forms such as tablets, capsules and oral suspensions and takes into consideration criteria derived from the concepts underlying the Biopharmaceutics Classification System, i.e. high solubility, high permeability for the active substance and high dissolution rate for the medicinal product. These criteria, along with a non-critical therapeutic range should be primarily considered; therefore the following characteristics have to be taken into account in order to justify the request for exemption from in vivo bioequivalence studies. Hence data must be supplied to justify the absence of such studies.

a) Characteristics related to the active substance:

i - risk of therapeutic failure or adverse drug reactions:

this risk depends on the requirements of special precautions with respect to precision and accuracy of dosing of the active substance, e.g. the need for critical plasma concentrations;

ii - risk of bioinequivalence:

evidence of bioavailability problems or bioinequivalence exists for some specific active substances;

iii - solubility:

When the active substance is highly water soluble, the product could be in general exempted from bioequivalence studies unless, considering the other characteristics, the exemption could entail a potential risk. Polymorphism and particle size are major determinants of dissolution rate and special attention should be paid to these characteristics. An active substance is considered highly water soluble if the amount contained in the highest dose strength of an immediate release product is dissolved in 250 ml of each of three buffers within the range of pH 1-8 at 37°C (preferably at or about pH 1.0, 4.6, 6.8);

iv - pharmacokinetic properties:

linear and complete absorption indicating high permeability reduces the possibility of an immediate release dosage form influencing the bioavailability.

b) Characteristics related to the medicinal product:

i - rapid dissolution

in case of exemption from bioequivalence studies, in vitro data should demonstrate the similarity of dissolution profile between the test product and the reference product in each of three buffers within the range of pH 1-8 at 37°C (preferably at or about pH 1.0, 4.6, 6.8). However, in cases where more than 85% of the active substance are dissolved within 15 minutes, the similarity of dissolution

profiles may be accepted as demonstrated (see appendix II);

ii - excipients

the excipients included in the composition of the medicinal product are well established and no interaction with the pharmacokinetics of the active substance is expected. In case of atypically large amounts of known excipients or new excipients being used, additional documentation has to be submitted;

iii - manufacture

the method of manufacture of the finished product in relation with critical physicochemical properties of the active substance (e.g. particle size, polymorphism) should be adequately addressed and documented in the development pharmaceutics section of the dossier.

5.1.2 Oral solutions

If the product is an aqueous oral solution at time of administration and contains an active substance in the same concentration as an oral solution currently approved as a medicinal product, no bioequivalence study is required, provided the excipients contained in it do not affect gastrointestinal transit, absorption or in vivo stability of the active substance.

In those cases where an oral solution has to be tested against an oral immediate release formulation a comparative bioavailability study will be required unless an exemption can be justified (see 5. 1. 1).

5.1.3 Non-Oral Immediate Release forms with systemic action

In general bioequivalence studies are required.

5.1.4 Modified Release and transdermal dosage forms

Requirements for bioequivalence studies in accordance with the specific guideline

5.1.5 Fixed combinations products

Combination products should in general be assessed with respect to bioavailability and bioequivalence of individual active substances either separately (in the case of a new combination) or as an existing combination. Criteria under 5.1.1 will apply to individual components. The study in case of a new combination should be designed in such a way that the possibility of a pharmacokinetic drug-drug interaction could be detected.

5.1.6 Parenteral solutions

The applicant is not required to submit a bioequivalence study if the product is to be administered as an aqueous intravenous solution containing the same active substance in the same concentration as the currently authorised product.

In the case of other parenteral routes, e.g. intramuscular or subcutaneous, if the product is of the same type of solution (aqueous or oily), contains the same concentration of the same active substance and the same or comparable excipients as the medicinal product currently approved, then bioequivalence testing is not required.

5.1.7 Gases

If the product is a gas for inhalation a bioequivalence study is not required.

5.1.8 Locally applied products

a) **Locally acting**

For products for local use (after oral, nasal, inhalation, ocular, dermal, rectal, vaginal etc. administration) intended to act without systemic absorption the approach to determine

bioequivalence based on systemic measurements is not applicable and pharmacodynamic or comparative clinical studies are in principle required. The lack of them should be justified (see specific Note for Guidance).

Whenever systemic exposure resulting from locally applied, locally acting medicinal products entails a risk of systemic adverse reactions, systemic exposure should be measured.

b) Systemically acting

For locally applied products with systemic action a bioequivalence study is always required.

5.2 In Vitro Dissolution

Dissolution studies are always necessary and consequently required. In vitro dissolution testing forms a part of the assessment of a bioequivalence waiver request based on criteria as described in section 5.1. Dissolution studies must follow the guidance as laid out in Appendix II.

5.3 Variations

If a product has been reformulated from the formulation initially approved or the manufacturing method has been modified by the manufacturer in ways that could be considered to impact on the bioavailability, a bioequivalence study is required, unless otherwise justified. Any justification presented should be based upon general considerations, e.g. as per 5.1.1, or on whether an acceptable in vivo / in vitro correlation has been established.

In cases where the bioavailability of the product undergoing change has been investigated and an acceptable correlation between in vivo performance and in vitro dissolution has been established, the requirements for in vivo demonstration of bioequivalence can be waived if the dissolution rate in vitro of the new product is similar to that of the already approved medicinal product under the same test conditions as used to establish the correlation (see Appendix II)

In all other cases bioequivalence studies have to be performed.

For variations of the innovator product the reference product for use in bioequivalence and dissolution studies is usually that authorised under the current formula, manufacturing method, packaging etc. and the product manufactured in line with the proposed changes is tested against this.

When variations to an essentially similar product are made the reference product for the bioequivalence study should be the innovator product.

5.4 Dose proportionality in immediate release oral dosage forms

If a new application concerns several strengths of the active substance a bioequivalence study investigating only one strength may be acceptable. However the choice of the strength used should be justified on analytical, pharmacokinetic and safety grounds. Furthermore all of the following conditions should be fulfilled:

- the pharmaceutical products are manufactured by the same manufacturer and process;
- the drug input has been shown to be linear over the therapeutic dose range (if this is not the case the strengths where the sensitivity is largest to identify differences in the two products should be used);
- the qualitative composition of the different strengths is the same;
- the ratio between amounts of active substance and excipients is the same, or, in the case of preparations containing a low concentration of the active substance (less than 5%), the ratio between the amounts of excipients is similar;

- the dissolution profile should be similar under identical conditions for the additional strengths and the strength of the batch used in the bioequivalence study.

If a new strength (within the approved dose range) is applied for on the basis of an already approved medicinal product and all of the stated conditions hold then a bioequivalence study is not necessary.

5.5 Suprabioavailability

If suprabioavailability is found, i.e. if the new product displays an extent of absorption appreciably larger than the approved product, reformulation to a lower dosage strength should be considered. In this case, the biopharmaceutical development should be reported and a final comparative bioavailability study of the reformulated new product with the old approved product should be submitted.

In case reformulation is not carried out the dosage recommendations for the suprabioavailable product will have to be supported by clinical studies. Such a pharmaceutical product should not be accepted as therapeutically equivalent to the existing reference product. If marketing authorisation is obtained, the new product may be considered as a new medicinal product.

To avoid confusion for both prescribers and patients, it is recommended that the name of suprabioavailable product precludes confusion with the older approved product

Suprabioavailable products cannot claim "essential similarity" (see section 2.5) with the innovator product.

APPENDIX I

Explanation of the symbols in paragraph 3.3

C_{max}: maximal plasma concentration;

C_{min}: minimal plasma concentration;

C_{av}: average plasma concentration;

t_{max}: time passed since administration at which the plasma concentration maximum occurs;

AUC_t: area under the plasma concentration curve from administration to last observed concentration at time t.

AUC_∞: area under the plasma concentration curve extrapolated to infinite time;

AUC_{t̄}: AUC during a dosage interval in steady state;

MRT: mean residence time;

A_{et}: cumulative urinary excretion from administration until time t;

A_{e∞}: cumulative urinary excretion extrapolated to infinite time;

t_{1/2}: plasma concentration half-life;

Fluctuation: $(C_{max} - C_{min})/C_{av}$

Swing: $(C_{max} - C_{min})/C_{min}$.

APPENDIX II

Dissolution testing

A medicinal product is composed of drug substance and excipients and the proportion between them, the type of excipients and the manufacturing method of the final product are chosen based on the content, the physicochemical and the bulk properties of the drug and on its absorption properties. Taken as a whole this gives each product certain dissolution characteristics.

During the development of a medicinal product a dissolution test is used as a tool to identify formulation factors that are influencing and may have a crucial effect on the bioavailability of the drug. As soon as the composition and the manufacturing process are defined a dissolution test is used in the quality control of scale-up and of production batches to ensure both batch-to-batch consistency and that the dissolution profiles remain similar to those of pivotal clinical trial batches. Furthermore, a dissolution test can be used to support the bioavailability of a new drug product, the bioequivalence of an essentially similar product or variations.

Therefore, dissolution studies can serve several purposes:

i - Quality assurance

- To get information on the test batches used in bioavailability/bioequivalence studies and pivotal clinical studies to support specifications for quality control.
- To be used as a tool in quality control to demonstrate consistency in manufacture
- To get information on the reference product used in bioavailability/bioequivalence studies and pivotal clinical studies

ii - Bioequivalence surrogate inference

- To demonstrate similarity between reference products from different Member States
- To demonstrate similarity between different formulations of an active substance (variations and new, essentially similar products included) and the reference medicinal product
- To collect information on batch to batch consistency of the products (test and reference) to be used as basis for the selection of appropriate batches for the in vivo study.

The test methodology should be in accordance with pharmacopoeial requirements unless those requirements are shown to be unsatisfactory. Alternative methods can be considered when justified that these are discriminatory and able to differentiate between batches with acceptable and non-acceptable performance of the product in vivo.

If an active substance is considered highly soluble, it is reasonable to expect that it will not cause any bioavailability problems if, in addition, the dosage system is rapidly dissolved in the physiological pH-interval expected after product administration. A bioequivalence study may in those situations be waived based on case history and similarity of dissolution profiles which are based on discriminatory testing, provided that the other exemption criteria in 5.1.1 are met. The similarity should be justified by dissolution profiles, covering at least three time points, attained at three different buffers (normally pH range 1-6.8; in cases where it is considered necessary pH range 1-8).

In the case of a drug or excipients that are insensitive to pH, profiles from only two buffer systems are required.

If an active substance is considered to have a low solubility and a high permeability, the rate limiting step for absorption may be dosage form dissolution. This is also the case when one or more of the excipients are controlling the release and subsequent dissolution step of the active

substance. In those cases a variety of test conditions is recommended and adequate sampling should be performed until either 90% of the drug is dissolved or an asymptote is reached. Knowledge of dissolution properties under different conditions e.g. pH, agitation, ionic strength, surfactants, viscosity, osmotic pressure is important since the behaviour of the solid system *in vivo* may be critical for the drug dissolution independent of the physico-chemical properties of the active substance. An appropriate experimental statistical design may be used to investigate the critical parameters and for the optimisation of such conditions.

Any methods to prove similarity of dissolution profiles are accepted as long as they are justified.

The similarity may be compared by model-independent or model-dependent methods e.g. by linear regression of the percentage dissolved at specified time points, by statistical comparison of the parameters of the Weibull function or by calculating a similarity factor e.g. the one defined below:

$$f_2 = 50 \cdot \log \left[\frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} [\bar{R}(t) - \bar{T}(t)]^2}{n}}} \right]$$

In this equation f_2 is the similarity factor, n is the number of time points, $R(t)$ is the mean percent drug dissolved of e.g. a reference product, and $T(t)$ is the mean percent drug dissolved of e.g. a test product.

The evaluation of similarity is based on the conditions of

- A minimum of three time points (zero excluded)
- 12 individual values for every time point for each formulation
- not more than one mean value of > 85% dissolved for each formulation
- that the standard deviation of the mean of any product should be less than 10% from second to last time point.

An f_2 value between 50 and 100 suggests that the two dissolution profiles are similar. In cases where more than 85% of the drug are dissolved within 15 minutes, dissolution profiles may be accepted as similar without further mathematical evaluation.

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International Psoriasis Community
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The National Institutes of Health ("NIH")

NIH is one of the foremost centers in the United States for biomedical research. NIH is divided into an extramural part, which handles biomedical research throughout the United States, and an intramural part, based mainly in Bethesda, Maryland. NIH is composed of various Institutes, each of which conducts research on health and disease. Studies involving volunteer subjects (research patients and healthy volunteers) are carried out in the Warren Grant Magnuson Clinical Center.

The Clinical Center is the federal government's premier biomedical research hospital, where clinical studies of the highest quality are performed. Because the research is funded by the federal government, there is no cost for care received at the Clinical Center.

Volunteer subjects at the Clinical Center become partners in a special relationship with members of the research teams in the search for better ways to treat disease.

What does it mean to be a volunteer?

The Patient Recruitment and Public Liaison Office at the NIH Clinical Center can answer your questions about current studies and how to participate. The health of millions has been improved because of advances in science and technology, and the willingness of thousands of individuals like you to take part in clinical research. The role of volunteer subjects as partners in clinical research is crucial in the quest for knowledge that will improve the health of future generations. Without your help, the research studies at the Clinical Center cannot be accomplished.

This site is designed help you understand what is involved in participating in clinical research. Before you take part in any study, we encourage you to read the material and be sure that you have satisfactory answers to the list of questions at the end. First decide which of the following categories best describes your situation:

Healthy volunteer:

A volunteer subject with no known significant health problems who participates in research to test a new drug, device, or intervention. A healthy volunteer may be a member of the community, an NIH investigator or other employee, or family members of a patient volunteer. Research procedures with healthy volunteers are designed to develop new knowledge, not to provide direct benefit to study participants.

Healthy volunteers have always played a vital role in medical research. We need to study healthy volunteers for several reasons: When developing a new technique such as a blood test or imaging device, we need healthy volunteers to help us define the limits of "normal." Healthy volunteers are recruited to serve as controls for patient groups. They are often matched to patients on such characteristics as age, gender, or family relationship. They are then given the same test, procedure, or drug the patient group receives. Investigators learn about the disease process by comparing the patient group to the healthy volunteers.

Some studies require a major commitment in time and effort on the part of the volunteer, or they may involve some discomfort. The research procedure may also carry some risk. The consent process for healthy volunteers involves a detailed discussion of all the study's procedures and tests. After this discussion, you will be given a consent document to read that describes the details of the study into which you are considering enrollment. You must read it carefully and sign it only if you understand what is involved and are prepared to accept the potential risk, discomforts, and inconvenience involved.

Patient volunteer:

A volunteer subject with a known health problem, who participates in research to better understand, diagnose, treat, or cure a particular disease or condition. Research procedures with a patient volunteer help develop new knowledge. Such procedures may or may not benefit individual study participants.

The following should help you better understand how the research is planned and carried out, how to weigh the risks, and how your safety and rights are safeguarded.

What is clinical research?

Patient volunteers have a particular illness or condition that can help research doctors and scientists better understand, diagnose, prevent, treat, or cure it. The research is planned to help others and may not benefit you directly. As a patient volunteer, you may be involved in studies similar to those described in the section on healthy volunteers. Benefits of such research may be indirect for you, but may help others.

These studies involve drugs, devices, or interventions designed to prevent, treat, or cure disease. It is important to remember, however, that although such studies may provide direct benefit to patient volunteers, the main aim is to prove, by scientific means, the effects and limitations of the experimental treatment. This may mean that some patients serve as controls by not taking the test drug, or they receive test doses of the drug large enough only to show that it is present, but not at a level that can treat the condition.

What is a clinical trial?

Clinical trials are a means of developing new treatments and medications for diseases and conditions. There are strict rules for clinical trials, which are monitored countrywide by the NIH and the FDA, especially when they involve new drugs. There are three types of study:

The *phase 1 study* is used to learn the "maximum tolerated dose" of a drug that does not produce unacceptable side effects. Patient volunteers are followed primarily for side effects, and not for how the drug affects their disease. The first few volunteer subjects receive low doses of the trial drug to see how the drug is tolerated and to learn how it acts in the body. The next group of volunteer subjects receives larger amounts. Phase 1 studies typically offer little or no benefit to the volunteer subjects.

The *phase 2 study* involves a drug whose dose and side effects are well known. Many more volunteer subjects are tested, to define side effects, learn how it is used in the body, and learn how it helps the condition under study. Some volunteer subjects may benefit from a phase 2 study.

The *phase 3 study* compares the new drug against a commonly used drug. Some volunteer subjects will be given the new drug and some the commonly used drug. The trial is designed to find where new drug fits in the managing a particular condition.

Determining the true benefit of a drug in a clinical trial is difficult. Medical research is dogged by the "placebo effect"--the real or apparent improvement in a patient's condition due to wishful thinking by the investigator or the patient. Medical techniques use three ways to rid clinical trials of this problem. These methods have helped discredit some previously accepted treatments and validate new ones. Methods used are the following: randomization, single-blind or double-blind studies, and the use of a placebo.

Randomization is when two or more alternative treatments are selected for you by chance, not by choice. The treatment chosen is given with the highest level of professional care and expertise and the results of each treatment are compared. Analyses are done at intervals during a trial, which may last years. As soon as one treatment is found to be definitely superior, the trial is stopped. In this way, the fewest number of patients receive the less beneficial treatment.

In *single-or double-blind studies*, the participants don't know which medicine is being used, and they can describe what happens without bias. Blind studies are designed to prevent anyone (doctors, nurses, or patients) from influencing the results. This allows scientifically accurate conclusions. In single-blind ("single-masked") studies, only the patient is not told what is being given. In a double-blind study, only the pharmacist knows; the doctors, nurses, patients, and other health care staff are not informed. If medically necessary, however, it is always possible to find out what the patient is taking.

Placebos are harmless, inactive substances made to look like the real medicine used in the clinical trial. Placebos

allow the investigators to learn whether the medicine being given works better or no better than ordinary treatment. In many studies, there are successive time periods, with either the placebo or the real medicine. In order not to introduce bias, the patient, and sometimes the staff, are not told when or what the changes are. If a placebo is part of a study, you will always be informed in the consent form given to you before you agree to take part in the study.

When you read the consent form, be sure that you understand what research approach is being used in the study you are entering. Remember, even with a clinical trial, there is no guarantee that the new approach will be effective for you.

Are there risks involved in participating in clinical research?

Risks are involved in clinical research, as in routine medical care and activities of daily living. In thinking about the risks of research, it is helpful to focus on two things: the degree of harm that could result from taking part in the study, and the chance of any harm occurring. Most clinical studies pose risks of minor discomfort, lasting only a short time. Some volunteer subjects, however, experience complications that require medical attention. In rare cases, volunteer subjects have received serious injuries or died of complications resulting from their participation in trials of experimental therapies.

The specific risks associated with any research protocol are described in detail in the consent document, which you are asked to sign before taking part in research. In addition, the major risks of participating in a study will be explained to you by a member of the research team, who will answer your questions about the study. Before deciding to participate, you should carefully weigh these risks. Although you may not receive any direct benefit as a result of participating in research, the knowledge developed may help others. The following section describes safeguards to protect the safety and rights of volunteer subjects.

How are my rights safeguarded?

Protocol review

As in any medical research facility, all new protocols produced at NIH must be approved by an institutional review board (IRB) before they can begin. The IRB, which consists of medical specialists, statisticians, nurses, social workers, and medical ethicists, is the advocate of the volunteer subject. The IRB will only approve protocols that address medically important questions in a scientific and responsible manner.

Informed consent

Your participation in any Clinical Center research protocol is voluntary. For every study in which you intend to participate, you will receive a document called "Consent to Participate in a Clinical Research Study" that explains the study in straightforward language. A member of the research team will discuss the protocol with you, explain its details, and answer your questions. Reading and understanding the protocol is your responsibility. You may discuss the protocol with family and friends. You will not be hurried into making a decision, and you will be asked to sign the document only after you understand the nature of the protocol and agree to the commitment. At any time after signing the protocol, you are free to change your mind and decide not to participate further. This means that you are free to withdraw from the study completely, or to refuse particular treatments or tests. Sometimes, however, this will make you ineligible to continue the study. If you are no longer eligible or no longer wish to continue the study, you will return to the care of the doctor who referred you to NIH.

Patient representative

The patient representative acts as a link between the patient and the hospital. The patient representative makes every effort to assure that patients are informed of their rights and responsibilities, and that they understand what the Clinical Center is, what it can offer, and how it operates. We realize that this setting is unique and may generate questions about the patient's role in the research process.

As in any large and complex system, communicating can be a problem and misunderstandings can occur. If you have an unanswered question or feel there is a problem you'd like to discuss, call the patient representative. The sooner your concerns are known, the easier they are to address.

Bill of Rights

Finally, whether you are a healthy or a patient volunteer subject you are protected by a bill of rights drawn up by the American Hospital Association for use in all hospitals in the country. The bill of rights concerns the care you receive, privacy, confidentiality, and access to medical records.

Questions to ask before agreeing to participate in a research protocol:

What is the purpose of the study?

What is required of me?

What is my role in the study-am I a healthy volunteer or a patient volunteer?

Will the study directly benefit me?

Will the study benefit others?

Are there risks? If so, what are they and what are the chances that they will occur?

What discomforts are involved?

What is the total time involved?

Are there other inconveniences?

Have I discussed participation in the study with those who are important to me, such as family and friends?

Do I wish to participate in this study?

For more information about the Clinical Center, contact CC Communications (OCCC@NIH.GOV) or call (301)-496-2563.

For more information on participating in research, contact the Patient Recruitment and Public Liason Office, prpl@cc.nih.gov or call 1-800-411-1222.

***Source of above information: NIH, USA

(13)



Patient Information Publications

NIH Clinical Center
National Institutes of Health

Partners in Research Volunteer Patients and the Clinical Center

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Some studies require a major commitment in time and effort on the part of the volunteer, or they may involve some discomfort. The research procedure may also carry some risk. The consent process for clinical research volunteers involves a detailed discussion of all the study's procedures and tests. After this discussion, he or she will be given a consent document to read. This document details the study being considered. It must be read carefully and signed only when the clinical research volunteer understands what is involved and is prepared to accept the potential risk, discomforts, and inconvenience involved.

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receives larger amounts. Phase 1 studies typically offer little or no benefit to the volunteer subjects.

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- *Randomization* is when two or more alternative treatments are selected by chance, not by choice. The treatment chosen is given with the highest level of professional care and expertise, and the results of each treatment are compared. Analyses are done at intervals during a trial, which may last

years. As soon as one treatment is found to be definitely superior, the trial is stopped. In this way, the fewest number of patients receive the less beneficial treatment.

- In *single- or double-blind studies*, the participants don't know which medicine is being used, and they can describe what happens without bias. Blind studies are designed to prevent anyone (doctors, nurses, or patients) from influencing the results. This allows scientifically accurate conclusions. In single-blind ("single-masked") studies, only the patient is not told what is being given. In a double-blind study, only the pharmacist knows; the doctors, nurses, patients, and other health care staff are not informed. If medically necessary, however, it is always possible to find out what the patient is taking.

- *Placebos* are harmless, inactive substances made to look like the real medicine used in the clinical trial. Placebos allow the investigators to learn whether the medicine being given works better or no better than ordinary treatment. In many studies, there are successive time periods, with either the placebo or the real medicine. In order not to introduce bias, the patient, and sometimes the staff, are not told when or what the changes are.

If a placebo is part of a study, you will always be informed in the consent form given to you before you agree to take part in the study.

When you read the consent form, be sure that you understand what research approach is being used in the study you are entering. Remember, even with a clinical trial, there is no guarantee that the new approach will be effective for you.

Are there risks involved in participating in clinical research?
Risks are involved in clinical research, as in routine medical care and activities of daily living. In thinking about the risks of research, it is helpful to focus on two things: the degree of harm that could result from taking part in the study, and the chance of any harm occurring. Most clinical studies pose risks of minor discomfort, lasting only a short time. Some volunteer subjects, however, experience complications that require medical attention. In rare cases, volunteer subjects have received serious injuries or died of complications resulting from their participation in trials of experimental therapies.

The specific risks associated with any research protocol are described in detail in the consent document, which you are asked to sign before taking part in research. In addition, the major risks of participating in a study will be explained to you by a member of the research team, who will answer your questions about the study. Before deciding to participate, you should carefully weigh these risks. Although you may not receive any direct benefit as a result of participating in research, the knowledge developed may help others. The following section describes safeguards to protect the safety and rights of volunteer subjects.

Protocol review

As in any medical research facility, all new protocols produced at NIH must be approved by an institutional review board (IRB) before they can begin. The IRB, which consists of medical specialists, statisticians, nurses, social workers, and medical ethicists, is the advocate of the volunteer subject. The IRB will only approve protocols that address medically important questions in a scientific and responsible manner.

Informed consent

Your participation in any Clinical Center research protocol is voluntary. For every study in which you intend to participate, you will receive a document called "Consent to Participate in a Clinical Research Study" that explains the study in straightforward language. A member of the research team will discuss the protocol with you, explain its details, and answer your questions. Reading the consent document and understanding the protocol is your responsibility. You may discuss the protocol with family and friends. You will not be hurried into making a decision, and you will be asked to sign the document only after you understand the nature of the protocol and agree to the commitment.

At any time after signing the consent document, you are free to change your mind and decide not to participate further. This means that you are free to withdraw from the study completely, or to refuse particular treatments or tests. Sometimes, however, this will make you ineligible to continue the study. If you are no longer eligible or no longer wish to continue the study, you will return to the care of the doctor who referred you to NIH.

Patient representative

The Patient Representative acts as a link between the patient and the hospital. The Patient Representative makes every effort to assure that patients are informed of their rights and responsibilities, and that they understand what the Clinical Center is, what it can offer, and how it operates. We realize that this setting is unique and may generate questions about the patient's role in the research process.

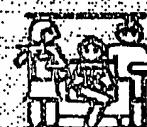
As in any large and complex system, communication can be a problem and misunderstandings can occur. If you have an unanswered question or feel there is a problem you'd like to discuss, call the Patient Representative. The sooner your concerns are known, the easier they are to address.

Bill of Rights

Finally, whether you are a clinical research or a patient volunteer subject, you are protected by the Clinical Center Patients' Bill of Rights. This document is adapted from the one made by the American Hospital Association for use in all hospitals in the country. The bill of rights concerns the care you receive, privacy, confidentiality, and access to medical records. Your patient handbook contains this information.

Questions to ask before agreeing to participate in a research protocol

1. What is the purpose of the study?
2. What is required of me?
3. What is my role in the study—am I a clinical research volunteer or a patient volunteer?
4. Will the study directly benefit me?
5. Will the study benefit others?
6. Are there risks? If so, what are they and what are the chances that they will occur?
7. What discomforts are involved?
8. What is the total time involved?
9. Are there other inconveniences?
10. Have I discussed participation in the study with those who are important to me, such as family and friends?
11. Do I wish to participate in this study?
12. When can I decide not to participate in all or part of the study? What will be the consequences?



2005

This information is prepared specifically for patients participating in clinical research at the Warren Grant Magnuson Clinical Center at the National Institutes of Health and is not necessarily applicable to individuals who are patients elsewhere. If you have questions about the information presented here, talk to a member of your healthcare team.

Where applicable, brand names of commercial products are provided only as illustrative examples of acceptable products, and do not imply endorsement by NIH, nor does the fact that a particular brand name product is not identified imply that such product is unsatisfactory.

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Questions about the Clinical Center?
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(14)

[\(http://www.open.mis.surrey.ac.uk/misweb/modules/7431.htm\)](http://www.open.mis.surrey.ac.uk/misweb/modules/7431.htm)

FULL MODULE DESCRIPTION

Module Name:	Short Name:	Module Title:	Conducting studies in non-patient volunteers
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Objectives/Learning Outcomes

The objectives of this module are to provide the candidate with a broad understanding of ethical and moral issues in conducting studies in healthy volunteers, including appropriate facilities, equipment and staff. The module will also cover the common toxicities of healthy volunteers, appropriate study design, administration and the most common adverse effects and their prevention/management with those reviewed.

Content

<ul style="list-style-type: none"> • National and international legal requirements and guidelines • Ethics of volunteer studies • Informed consent • Staff training and experience • Appropriate facilities • Common adverse reactions and the management of acute medical emergencies • Definition of a healthy volunteer, appropriate study design and considerations of the limits of normality for laboratory data and muscular data • Assessing animal toxicology and pharmacokinetic data • Design of healthy volunteer studies • Conduct of studies with radiolabelled drugs • Studies in special populations (young, elderly, pre- and post-menopausal women, pregnant lactating)
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Prerequisites

Module

Pattern of Delivery

4 continuous days of contact to a minimum of 27 hrs followed by self-directed learning and written assignments for a minimum of 6 hrs and 10 hours of study per module.

Methods of Teaching/Learning

Other than active contact (i.e. presentations) with some group exercises.

Methods of Assessment and Weighting

A variety of problem-based exercises that require personal research and synthesis of concepts.

Selected Texts

- Eds. Chubrik, S. & Mair, J. 1991. *Principles of Formulation and Drug Development*. London: Clinical Pharmacology. John Wiley. ISBN 0-471-92551-1
- Eds. Giese, S. & Gmelin, R. 1996. *Handbuch der Pharmazie*. 11. Auflage. Berlin: Deutscher Apotheker Verlag. ISBN 3-8047-0991-1
- Eds. Grady, J. & Joubert, P.H. 1997. *Handbook of Practical Clinical Formulation*. London: Macmillan Press. ISBN 0-333-48777-1
- Eds. Grady, J. & Joubert, P.H. 1997. *Handbook of Practical Clinical Formulation*. CRC Press. ISBN 0-8493-3210-6

Endorse 1

(15)

U.S. Food and Drug Administration
Equality In Clinical Trials Drugs and Gender
by Judith Levine Willis FDA Consumer Special Report

Women woke up almost twice as fast from anesthesia as men in a study reported at the annual meeting of the American Society of Anesthesiologists, in October 1996. In another recent study, the class of painkillers called kappa opoiods seemed to work about twice as well for women as for men. These are but two of a growing number of clinical studies providing new information about possible differences in ways women and men respond to drugs.

Reporting on the anesthesia study, one of the researchers, Peter S. Glass of Duke University Medical Center, said the gender finding emerged unexpectedly during a study of how management of anesthetic drugs during surgery influences recovery time from general anesthesia. The study included 300 people. Women took an average of seven minutes to emerge from anesthesia, while men took 13. The difference occurred independently of differences in body weight.

In the painkiller study, according to a report published in the November 1996 issue of *Nature Medicine*, women having wisdom teeth removed had a better and longer response to these drugs than men, even when factors such as body size and menstrual cycle were considered. Until recently, women were not routinely included in many human trials to determine whether drugs are safe and effective. The reason usually given was that excluding women protected them, since there was often no way to be sure that a woman was not pregnant or that the drug might not cause some problem that might interfere with future pregnancies. In addition, it was thought that women's hormonal cycling or other factors peculiar to being female might constitute variables that could skew trial results.

However, over the last several years, research has accumulated indicating that drug results from male-only trials may not apply equally to women, or may not give data on effects important to women. At one time, researchers thought most of the differences between the way men and women reacted to drugs might be attributed to differences in height, weight and hormones. This meant, for example, that simply because most men weigh more than most women, most men would be able to tolerate higher doses of medications without side effects. Today many scientists think it's far more complicated. For example, the liver may be different enough in men and women to account for at least part of why most women seem to metabolize drugs differently than men. And in the case of pain relief, there may be gender differences in pain tolerance and differences in the way each gender responds to various pain medications.

The rising recognition of these and other gender factors has brought changes in the way the Food and Drug Administration asks firms to test drugs and in the data the agency asks them to provide.

For example, in September 1995 FDA proposed to amend its investigational and new drug regulations to require drug sponsors to include data about gender, as well as age and race. The proposal does not require manufacturers to conduct additional studies. Rather, the manufacturers would simply provide information previously gathered in a new, more useful, format.

FDA suggested changes of this nature in 1993 as a revision to a 1977 Guideline, "General Considerations for Clinical Evaluation of Drugs," after the agency found that few women were included in the earliest stages of drug clinical trials. In addition, the agency found that there had been little study of the effects on drug action of such factors as the menstrual cycle, menopause, and hormone use. The 1993 guideline left gender analysis voluntary; it was not a requirement.

Subsequent studies by FDA and the General Accounting Office have shown that women are often included in later phases of clinical trials, and are included in proportions similar to the proportion of women who have the condition the drug is being tested for. But FDA believes

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that inclusion alone is not enough. What is needed, in addition, is an effort to use data from the trials to discover potential gender differences.

Impact of HIV

Rachel Behrman, M.D., supervisory medical officer in FDA's division of antiviral drug products, says the issues surrounding these changes were brought to the forefront by efforts to treat HIV (the virus that causes AIDS) in women.

"The guidelines always provided that women with serious or life-threatening diseases could obtain an experimental drug in early phases of testing," says Behrman. "What's new is that now we're saying to drug manufacturers, 'Not only do we recommend that you study it in women, we may insist that you do so, if it's going to be used by women who have serious and life-threatening diseases.'"

She explains that because of the urgent need, drug testing for HIV is on a fast track, with condensed stages of controlled studies and, frequently, fewer people in them.

"Since the process moves so quickly, extensive clinical data collected over a long period of time often are not available," she says. "You need to know as soon as possible, for example, what adverse effects occur that might be dose-dependent, and work up those differences early in the drug development program. You don't want to wait for later stages of testing to begin to define dosage adjustments for men and women."

Many researchers feel the reason for including women in any phase 1 drug trial --- regardless of the seriousness of the disease --- should be to provide important data about the drug. This data includes whether women in general absorb, metabolize or excrete the drug differently from men, or have different reactions to the drug.

Early in the AIDS epidemic, very few women with HIV were included in studies. But their numbers are increasing. According to the National Institute of Allergy and Infectious Diseases (NIAID), in 1995 women accounted for 16.2 percent of adult participants in the AIDS Clinical Trials Group, the institute's largest clinical trials network. That percentage is up from 7 percent in 1988 and 8.4 percent in 1991.

In a study by FDA's Behrman, Kimberly Struble, Theresa Toigo and Debra Birnkrant, 136 of 156 clinical studies of HIV treatment conducted between 1988 and 1994 enrolled women. And, even in the 20 trials that did not include women, enrollment of women --- including those of childbearing ages --- was permitted. Enrollment of women in the other trials ranged up to 64 percent, with a mean of 11.6 percent.

Some HIV studies include only women. Better information on the length of survival and quality of life in HIV-infected women are research goals of the Women's Interagency HIV Study, conducted by the Centers for Disease Control and Prevention and NIAID. This large-scale study is designed to identify clinical signs of HIV infection in women, describe how the immune system declines, and look for factors that can affect the progression of the disease. It will also examine factors influencing women's access to health care.

Other NIAID-sponsored studies center on pregnancy and HIV. They are designed to examine the effect of antiviral drug treatment on both the mother and fetus, the influence of HIV on pregnancy, and the effects of pregnancy on the course of HIV infection. In addition, two experimental vaccines are being tested in pregnant women who are infected with HIV but are otherwise healthy. Phase 1 studies focus primarily on safety, but will also evaluate the vaccines' potential to reduce the amount of virus in the mother and to stimulate antibodies that will prevent infection of the fetus.

In August 1994, FDA approved the antiviral Retrovir (zidovudine, or AZT) to help prevent maternal-fetal transmission of HIV. A study of the drug for this use was halted early when preliminary data showed extremely encouraging results; that 8.3 percent of babies born to HIV-infected pregnant women treated with the drug became infected, compared with 25.5 percent of babies born to women on placebo.

Marian Segal also contributed to this article.

Your Guide to Women's Health, Third Edition, an FDA Consumer Special Report, September 1997. <http://www.fda.gov/oashi/aids/equal.html>

Endorse 2

16 I Chromatogr. 1986 Oct 31;382:247-52.

Analysis of homocysteine in human urine using high-performance liquid chromatography with electrochemical detection.

Thomson SB, Tucker DJ.

PIP: This report describes the modifications made to the analytical system prior to determination of homocysteine in human urine. It also contains data which compare the homocysteine concentrations in the urine of males and females as well as the results of a study in which the effect of oral contraceptive (OC) use by a group of women upon their urinary concentrations was compared to a control group. Furthering this work, the effect of cigarette smoking on the urinary homocysteine concentrations of OC users as compared to control was assessed as were the urinary homocysteine concentrations over a 28-day collection period for both an OC-using female and a male volunteer. 2M perchloric acid (AR grade, Ajax Chemicals) was used to prevent thiol group oxidation. Approximately 50 ml of urine flow from volunteers participating in this study were collected into vessels containing 10 ml of 2M perchloric acid. In all cases the samples were obtained in the morning immediately upon waking, and for the comparison of OC users with controls, the urine samples were obtained on the 21st day of their cycle. Homocysteine was quantified by the standard addition technique using peak-height measurement, the determination being carried out in triplicate. The homocysteine concentration then was expressed per mg creatinine. All the study participants were apparently healthy with an average age in the mid-20s for the females and the late 20s for the males. The significantly higher excretion rate of homocysteine for the males compared with the females was of interest since the risk of myocardial infarction for males is up to 6 times greater than for females in the under 40 age bracket. Neither OC formulation caused an increase in the excreted level of homocysteine in the urine of users as compared to the control group. It seems that the increased incidence of thrombotic episodes observed among women using OCs as compared to controls was due to a cause other than an elevated homocysteine level caused by the synthetic steroid components of the OC formulation. The similarity of homocysteine excretion levels in the urine of smoking and nonsmoking OC users suggests that smoking most likely increases the risks associated with OC, including that of developing thrombosis. Other results show that any homocysteine urinary excretion changes which might follow the estrogen pattern of a triphasic OC formulation during a cycle would appear to be impossible to detect. This is because dietary effects, among other things, may cause sufficiently large changes in the level of homocysteine excreted in the urine to mask any effects upon the excretion rate that hormonal changes might produce.

17 Scand J Clin Lab Invest. 1992 Jun;52(4):283-7.

Plasma homocysteine in women on oral oestrogen-containing contraceptives and in men with oestrogen-treated prostatic carcinoma.

Brattstrom L, Israelsson B, Olsson A, Andersson A, Hultberg B.

The mechanism by which oral oestrogen-containing contraceptives in women and oestrogen treatment of prostatic carcinoma in men increases the risk of vascular disease is unclear. These agents decrease serum concentrations of vitamin B12, pyridoxal 5-phosphate, and folate, all of which are essential for the metabolism of the atherogenic amino acid homocysteine. We found serum vitamin B12 concentrations to be lower in 17 women using oral contraceptives (219 +/- 84 pmol l-1) than in 13 age-matched female controls (385 +/-

129, p less than 0.001), but similar values were obtained in the two groups both for fasting plasma homocysteine concentrations (9.1 +/- 2.4 vs 9.2 +/- 3.6 μ mol l-1) and for the increase in these concentrations after methionine loading (19.2 +/- 7.5 vs 17.8 +/- 5.2 μ mol l-1). In five men with prostatic carcinoma, high-dose oestrogen treatment decreased serum vitamin B12 concentrations by a mean of 30% (p less than 0.05) within 4 weeks, during which fasting plasma homocysteine concentrations decreased (13.8 +/- 4.5 vs 10.5 +/- 2.8 μ mol l-1) and response to methionine loading increased (12.4 +/- 3.4 vs 17.3 +/- 5.1 μ mol l-1), though the latter changes were non-significant. Our findings do not support the hypothesis that hyperhomocystinemia explains cardiovascular risk in women using oral oestrogen-containing contraceptives, or in oestrogen-treated men with prostatic carcinoma.

Obstet Gynecol. 1999 Oct;94(4):485-91.

Hormone replacement therapy and plasma homocysteine levels.

van Baal WM, Smolders RG, van der Mooren MJ, Teerlink T, Kenemans P.

OBJECTIVE: To compare the effects of 4 and 12 weeks of combined estradiol-progestogen replacement with unopposed estradiol therapy on fasting plasma total homocysteine concentrations in healthy postmenopausal women. **METHODS:** In this prospective, 12-week study in healthy postmenopausal women, we randomly assigned 59 women to sequentially combined daily 2 mg estradiol (E2) plus either trimegestone 0.5 mg daily or dydrogesterone 10 mg daily (n = 28), or to unopposed daily 2 mg estradiol (n = 16), or to placebo (n = 15).

RESULTS: Fasting plasma total homocysteine concentrations decreased by 9.4% in the combined estradiol-progestogen group and by 5.1% in the estradiol-only group, and they increased by 2.4% in the placebo group (analysis of covariance: combined hormone replacement therapy compared with placebo ($P = .02$), combined therapy compared with estradiol ($P = .23$); and estradiol compared with placebo ($P = .26$)). Reductions were detectable after 4 weeks of combined estradiol-progestogen treatment. The data suggest an additional progestogen-related reduction in homocysteine levels of 0.7 μ mol/L and 0.4 μ mol/L after 4 and 12 weeks, respectively. Women with a baseline homocysteine concentration in the highest quartile had significantly greater reductions in homocysteine compared with women with an initial homocysteine value in the lowest quartile.

CONCLUSION: Fasting total homocysteine concentrations were significantly reduced by combined estradiol-progestogen replacement. Women with high homocysteine levels at baseline benefit the most. The progestogens used in this study did not have an unfavorable effect on homocysteine metabolism.

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Scand J Clin Lab Invest 1992; 52: 283-287

Plasma homocysteine in women on oral oestrogen-containing contraceptives and in men with oestrogen-treated prostatic carcinoma

L. BRATTSTRÖM,* B. ISRAELSSON,† A. OLSSON,‡ A. ANDERSSON§

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University of Lund, Departments of *Neurology, †Urology, and §Clinical Chemistry, University Hospital, Lund, and the ‡Department of Medicine, Malmö General Hospital, Sweden.

Brattström L, Israelsson B, Olsson A, Andersson A, Hultberg B. Plasma homocysteine in women on oral oestrogen-containing contraceptives and in men with oestrogen-treated prostatic carcinoma. Scand J Clin Lab Invest 1992; 52: 283-287.

The mechanism by which oral oestrogen-containing contraceptives in women and oestrogen treatment of prostatic carcinoma in men increases the risk of vascular disease is unclear. These agents decrease serum concentrations of vitamin B₁₂, pyridoxal 5-phosphate, and folate, all of which are essential for the metabolism of the atherogenic amino acid homocysteine. We found serum vitamin B₁₂ concentrations to be lower in 17 women using oral contraceptives ($219 \pm 84 \text{ pmol l}^{-1}$) than in 13 age-matched female controls ($385 \pm 129 \text{ pmol l}^{-1}$), but similar values were obtained in the two groups both for fasting plasma homocysteine concentrations ($9.1 \pm 2.4 \text{ vs } 9.2 \pm 3.6 \text{ } \mu\text{mol l}^{-1}$) and for the increase in these concentrations after methionine loading ($19.2 \pm 7.5 \text{ vs } 17.8 \pm 5.2 \text{ } \mu\text{mol l}^{-1}$). In five men with prostatic carcinoma, high-dose oestrogen treatment decreased serum vitamin B₁₂ concentrations by a mean of 30% ($p < 0.05$) within 4 weeks, during which fasting plasma homocysteine concentrations decreased ($13.8 \pm 4.5 \text{ vs } 10.5 \pm 2.8 \text{ } \mu\text{mol l}^{-1}$) and response to methionine loading increased ($12.4 \pm 3.4 \text{ vs } 17.3 \pm 5.1 \text{ } \mu\text{mol l}^{-1}$), though the latter changes were non-significant. Our findings do not support the hypothesis that hypethomocysteinaemia explains cardiovascular risk in women using oral oestrogen-containing contraceptives, or in oestrogen-treated men with prostatic carcinoma.

Key words: folate, vascular disease, vitamin B₁₂

Lars Brattström, MD, Neurology Section, Department of Medicine, County Hospital, S-391 85 Kalmar, Sweden.

Although the risk for cardiovascular disease is increased both in women using oestrogen-containing oral contraceptive agents (OCAs) and in oestrogen-treated men with prostatic carcinoma [1, 2], no specific factor has been identified to explain why OCAs and oestrogen treatment are associated with reduced serum concentrations of both vitamin B₁₂, folate, and pyridoxal 5-phosphate (PLP, the active co-enzymatic form of vitamin B₆) [3-9]. These vitamins are essential co-factors in the metabolism of the atherogenic amino acid homocysteine

[10]. Deficiency of any of these vitamins results in increased plasma homocysteine [11-13]. Several studies have shown plasma homocysteine to be increased in occlusive arterial disease, and hyperhomocysteinaemia is proposed to be an atherogenic risk factor [14]. In some of these studies, concentrations of the above vitamins have been found to be inversely correlated to those of plasma homocysteine. Supplementation with folic acid and pyridoxine (vitamin B₆) has been found to normalize increased plasma homocysteine concentrations [14].

Although urinary excretion of homocysteine has been found to be considerably increased in oestrogen-treated rats, that in women on oestrogen-containing OCAs was similar to that in women not on OCAs [15, 16]. However,

both in oestrogen-treated rats and in women on oestrogen-containing OCAs, tolerance to methionine (the precursor of homocysteine) was decreased, as reflected by increased numbers of detached endothelial cells in blood (i.e., indicating the presence of vascular injury) [17]. The decrease in methionine tolerance was found to be prevented by the administration of pyridoxine [17]. Thus, there are both theoretical and experimental indications that, at least in part, the increased cardiovascular risk in OCA users and oestrogen-treated prostatic carcinoma patients may be mediated by increased plasma homocysteine [18].

Therefore, we studied plasma homocysteine concentrations both in the fasting state and after methionine loading in young women using and not using oestrogen-containing OCAs, and in men with prostatic carcinoma before and 4 weeks after the start of high-dose oestrogen.

METHODS

Subjects

The women investigated were 17 20-29-year-old women (23.4 ± 2.7 years, mean ± SD) who had used oestrogen-containing OCAs regularly for at least 2 years, and 13 18-28-year-old women (23.8 ± 2.9 years) who had not used OCAs for the preceding two years. Both groups were student volunteers in subjectively excellent health. The OCAs used were either a triphasic

formulation containing 30-40 µg of ethynodiol diacetate and 50-125 µg of levonorgestrel, or a formulation containing 30 µg of ethynodiol diacetate and 150 µg of levonorgestrel.

The men investigated were 65-75-year-old men (70.1 ± 2.8 years) with newly diagnosed prostatic carcinoma. They were otherwise healthy and not on any medication at the start of the study. They were investigated before and 4 weeks after administration of 160 mg of polystyrene phosphate intramuscularly. During the 4-week period, the patients were also given ethynodiol diacetate orally at a dose of 1 mg daily for the first 14 days and then 150 µg daily.

Methods

At 8 am, fasting venous blood samples were drawn for measurements of creatinine, vitamin B₁₂ and albumin in serum, folate in blood, and homocysteine and methionine in plasma. L-methionine (3.8 g m⁻² body surface area) was then administered orally in about 200 ml of fruit juice, after which the subjects ate a standardized breakfast consisting of coffee or tea and two cheese sandwiches. Four hours after methionine loading, a second sample was collected for plasma amino acid measurements.

Blood samples for measurements of homocysteine and methionine in plasma were drawn in evacuated tubes containing EDTA, immediately placed on ice, and centrifuged within 15 min at 3000 g and +4 °C for 5 min. The plasma was separated and stored at -70 °C until analysed. Total plasma homocysteine (the sum of all homocysteinylin moieties, whether in sulphhydryl or disulphide forms, free or protein-bound) and methionine were measured as previously described [19]. Blood samples collected for measurements of the remaining biochemical variables were sent to the hospital biochemical laboratory for routine analysis.

DISCUSSION

The present OCA users had manifestly lower serum vitamin B₁₂ concentrations and a tendency toward lower blood folate concentrations

TABLE I. Plasma homocysteine and some other variables in women using or not using oestrogen-containing OCAs and in men with prostatic carcinoma before and after 4 weeks of high-dose oestrogen treatment (means ± SD)

	Women		Oestrogen treatment of men	
	-OCAs	+OCAs	Before	After
Number	13	17	5	5
Serum creatinine (µmol l ⁻¹)	80.4 ± 9.1	82.5 ± 7.7	79.8 ± 17.0	74.4 ± 12.3
Serum albumin (g l ⁻¹)	41.3 ± 2.2	39.2 ± 2.1**	34.2 ± 4.1	30.8 ± 2.5
Blood folate (nmol l ⁻¹)	340 ± 145	267 ± 64	305 ± 123	297 ± 102
Serum vitamin B ₁₂ (pmol l ⁻¹)	385 ± 129	219 ± 84**	321 ± 161	225 ± 101*
Plasma homocysteine (µmol l ⁻¹)				
Fasting	9.2 ± 3.6	9.1 ± 2.4	13.8 ± 4.5	10.5 ± 2.8
Post-load increase	17.8 ± 5.2	19.2 ± 7.5	12.4 ± 3.4	17.3 ± 5.1
Fasting	23.6 ± 3.5	23.9 ± 1.2	25.6 ± 2.9	24.8 ± 3.3
Post-load	714 ± 88	771 ± 128	797 ± 104	811 ± 158

*p<0.05 significant difference compared with before oestrogen treatment; **p<0.01. ***p<0.001 significant difference compared with women not using OCA.

RESULTS

The results are summarized in Table I. Women using oestrogen-containing OCAs had on average 5% lower serum albumin and 43% lower serum vitamin B₁₂ concentrations than women not using OCAs. These differences were significant. There was also a tendency toward lower blood folate concentrations in OCA users than in non-OCA users. After neither fasting nor post-methionine loading were there any differences in plasma homocysteine or methionine values between the two groups of women.

Oestrogen treatment of men with prostatic carcinoma resulted in decreased serum vitamin B₁₂ concentrations in all five patients, the mean decrease of 30% being significant. Although the oestrogen treatment resulted in a 24% reduction in the mean baseline (i.e., pre-load) plasma homocysteine concentration, and a 40% increase in the homocysteine response to methionine loading, these differences did not reach statistical significance. The oestrogen treatment had no effect on plasma methionine concentrations, either fasting or post-load.

Statistics

Results are expressed as means and standard deviations (SD). In assessing statistical significance, Student's *t*-test was used when two groups were compared and a paired *t*-test when paired were compared. The *p* values are two-tailed, and a *p* value of less than 0.05 was considered significant.

DISCUSSION

The present OCA users had manifestly lower serum vitamin B₁₂ concentrations and a tendency toward lower blood folate concentra-

tions than non-OCA users, findings consistent with those of previous studies [3-5]. Both vitamins serve as co-factors for the methylation of homocysteine to methionine and deficiency of either vitamin results in hyperhomocysteinaemia [11-13]. Even in non-deficient subjects, the concentrations of these vitamins are related to that of plasma homocysteine [14]. Nonetheless, the present OCA users and non-OCA users had similar plasma concentrations both of homocysteine and methionine, both fasting and after methionine loading. This suggests that the effects of oestrogen-containing OCAs on the levels of these vitamins are of no functional importance for the metabolism of homocysteine and methionine. There have been several reports of low plasma pyridoxal 5-phosphate concentrations in OCA users [6-9]. However, in other studies urinary excretion of cystathione and homocysteine, both indicators of pyridoxal 5-phosphate deficiency, did not differ between OCA users and non-OCA users either before or after methionine loading [20, 21], and these results are consistent with ours in showing no effect of OCAs on homocysteine metabolism, though pyridoxal 5-phosphate was not measured in the present study.

Plasma homocysteine concentrations have been reported to be markedly lower in pregnant women than in non-pregnant women [22], and to be only about 50% of normal in the vast majority of women using OCAs [23], though the

Plasma homocysteine concentrations have been reported to be markedly lower in pregnant women than in non-pregnant women [22], and to be only about 50% of normal in the vast majority of women using OCAs [23], though the

latter finding is not in agreement with our results. Also in the present five men with prostatic carcinoma, we observed a significant vitamin B₁₂ lowering effect of oestrogen treatment. In each case, the serum vitamin B₁₂ concentration decreased from 20% to 39%. Concomitantly, the mean baseline (i.e. pre-load) plasma homocysteine concentration decreased and the post-load plasma homocysteine response to methionine loading increased, though these changes were non-significant. We also measured baseline plasma homocysteine in five other men with prostatic carcinoma, before and 4 weeks after bilateral ablation testis, but found no changes in homocysteine concentrations (unpublished observation). The only consistent change found in the present study, confirming those of previous studies [3-5, 24], was that both low-dose (OCAs) and high-dose oestrogen treatment (prostatic carcinoma) reduced the serum vitamin B₁₂ concentration. The response to oestrogen would not appear to be dose-dependent [3]. The mechanism by which serum vitamin B₁₂ is reduced is not fully understood. Deficiency of haptocorrin, the main vitamin B₁₂ binder in serum, has been suggested to be the most likely explanation. Haptocorrin deficiency results in 'false low' serum vitamin B₁₂ concentrations, but not in clinical vitamin B₁₂ deficiency [3]. There is evidence that both vitamin B₁₂ reserves, Schilling test results and urinary methylmalonic acid excretion are normal in OCA users [3, 25], all being consistent with our findings of normal plasma homocysteine concentrations but reduced serum vitamin B₁₂ concentrations.

To sum up, the present study offers no evidence that oral oestrogen-containing OCAs users or men treated with high-dose oestrogen for prostatic carcinoma have increased plasma homocysteine concentrations which might explain their higher incidence of vascular disease. However, it was very recently reported that use of oestrogen-containing OCAs cause recurrent periods of hyperhomocysteinaemia; a finding considered a possible predisposition to vascular disease [26].

Diseases, the Swedish Medical Research Council (grant no. B90-19X-091995-01A), the Albert Pahsson Foundation, and the Foundation for Stroke Research.

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129, p less than 0.001), but similar values were obtained in the two groups both for fasting plasma homocysteine concentrations (9.1 +/- 2.4 vs 9.2 +/- 3.6 mumol l-1) and for the increase in these concentrations after methionine loading (19.2 +/- 7.5 vs 17.8 +/- 5.2 mumol l-1). In five men with prostatic carcinoma, high-dose oestrogen treatment decreased serum vitamin B12 concentrations by a mean of 30% (p less than 0.05) within 4 weeks, during which fasting plasma homocysteine concentrations decreased (13.8 +/- 4.5 vs 10.5 +/- 2.8 mumol l-1) and response to methionine loading increased (12.4 +/- 3.4 vs 17.3 +/- 5.1 mumol l-1), though the latter changes were non-significant. Our findings do not support the hypothesis that hyperhomocysteinemia explains cardiovascular risk in women using oral oestrogen-containing contraceptives, or in oestrogen-treated men with prostatic carcinoma.

18
Obstet Gynecol. 1999 Oct;94(4):485-91.
Hormone replacement therapy and plasma homocysteine levels.

van Baal WM, Smolders RG, van der Mooren MJ, Teerlink T, Kenemans P.

OBJECTIVE: To compare the effects of 4 and 12 weeks of combined estradiol-progestogen replacement with unopposed estradiol therapy on fasting plasma total homocysteine concentrations in healthy postmenopausal women. **METHODS:** In this prospective, 12-week study in healthy postmenopausal women, we randomly assigned 59 women to sequentially combined daily 2 mg estradiol (E2) plus either trimegestone 0.5 mg daily or dydrogesterone 10 mg daily (n = 28), or to unopposed daily 2 mg estradiol (n = 16), or to placebo (n = 15).

RESULTS: Fasting plasma total homocysteine concentrations decreased by 9.4% in the combined estradiol-progestogen group and by 5.1% in the estradiol-only group, and they increased by 2.4% in the placebo group (analysis of covariance: combined hormone replacement therapy compared with placebo (P = .02), combined therapy compared with estradiol (P = .23), and estradiol compared with placebo (P = .26). Reductions were detectable after 4 weeks of combined estradiol-progestogen treatment. The data suggest an additional progestogen-related reduction in homocysteine levels of 0.7 micromol/L and 0.4 micromol/L after 4 and 12 weeks, respectively. Women with a baseline homocysteine concentration in the highest quartile had significantly greater reductions in homocysteine compared with women with an initial homocysteine value in the lowest quartile.

CONCLUSION: Fasting total homocysteine concentrations were significantly reduced by combined estradiol-progestogen replacement. Women with high homocysteine levels at baseline benefit the most. The progestogens used in this study did not have an unfavorable effect on homocysteine metabolism.

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YUGOSLAVIA

Sonographic detection of physiologic pleural fluid in normal pregnant women.
Kodlancic I, Pusemajak S, Kodlancic K, Vidmar G.

PURPOSE: This prospective study was conducted to determine the normal incidence of a small amount of pleural fluid in healthy pregnant women on sonography. **METHODS:** Chest sonography to detect pleural fluid was performed in 47 pregnant volunteers, first with the subjects leaning on 1 elbow (the "elbow position") and then with subjects in a sitting position. A 9-12-MHz linear-array probe was used. If the result of this first examination was positive (showing a 2-mm or thicker anechoic layer), the subject was rescanned with a 3-6-MHz large-radius curved-array probe. **RESULTS:** A thin layer of fluid with a typical wedge-shaped appearance was visible in the pleural cul de sac space of 28/47 (59.5%) pregnant volunteers, on both sides in 18/47 (38.3%) and unilaterally in 10/47 (21.2%). The mean (+/- standard deviation) thickness of fluid layer (mean of measurements obtained in both positions) was 2.9 +/- 1.1 mm (range, 1.8-6.4 mm). The number of pregnant women with visible pleural fluid was the same regardless of the fetus' sex, but the difference in fluid layer thickness between the 2 groups was statistically significant (by the t-test, $p < 0.05$). A fluid layer thicker than 3 mm was easily detected with the 3-6-MHz abdominal curved-array probe in 7/47 (15%) women. **CONCLUSIONS:** Small amounts of pleural fluid can be visualized by chest sonography in healthy pregnant women. This result, if isolated, should not be taken as a sign of occult thoracic disease.

Dev Med Child Neurol. 1990 Dec;32(12):1058-60.

Incidence of inguinal hernia in children with congenital cerebral palsy.
Reimers JL, Latorcha JE.

(19) The incidence of inguinal hernia among 247 children with cerebral palsy was ascertained. During the first year of life, 20 of the 153 boys developed hernia, as did one of the 94 girls. Among boys with birthweights of 1000 to 2000g the incidence was 31 per cent, which is twice the rate for normal children. The incidence among boys with birthweights greater than 2000g was 8 per cent. A comparison group could not be found, but this incidence appears to be excessive, considering the reported normal incidence of 1 to 4 per cent in boys. The authors recommend routine examination for inguinal hernia, particularly for boys with birthweights less than 2000g and with spastic tetraplegia. Conversely, one should be alert to the possibility of concurrent spasticity in preterm males with inguinal hernia.

Eur J Echocardiogr. 2005 Jul 18; [Epub ahead of print]

What is a "NORMAL" right ventricle?
Kovalova S, Necas J, Vespalac J.

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AIMS: The aim of this project was to define NORMAL values of right ventricular (RV) volumes and ejection fraction (EF) in HEALTHY POPULATION using 2D-echocardiography. **METHODS AND RESULTS:** The "patient" group comprised 91 healthy volunteers aged 17-62 years. RV volumetry was based on ellipsoidal shell model method. Left ventricular (LV) volumes were assessed by Teichholz formula. All volumes were indexed per m^2 of BSA and the rate distribution of measured and calculated values were evaluated. The NORMAL range of individual parameters was expressed as mean value +/- 2 standard deviations (delta). A pair test was used to compare corresponding results of the RV and LV measurements. The regression analysis was used to test the relationship between LV and RV volumes and age. Indexed enddiastolic and endsystolic RV volumes were $79.1 \pm 29.9 \text{ ml}$ and $32.6 \pm 19.7 \text{ ml}$, respectively, EF being $50 \pm 9.7\%$ in men and $58 \pm 13.6\%$ in women. No correlation with patient's age was observed. **CONCLUSION:** Enddiastolic and endsystolic volumes of RV were significantly higher than those of LV. EF of RV was lower as compare to LV. Right ventricular EF in men was lower than that in women. There was no correlation between EF and patient's age.

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IN THE U.S. PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF APPEALS AND INTERFERENCES

Inventor Gabor BOGYE
Patent App. 09/890,029
Filed 24 July 2001 Conf. No. 6045
For PHARMACEUTICAL COMBINATION OF PROGESTERONE AND
FOLIC ACID
Art Unit 1617 Examiner Hui, S

Appeal filed 29 September 2005

Hon. Commissioner of Patents
Box 1450
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DOCUMENT REQUEST FOR REFUND

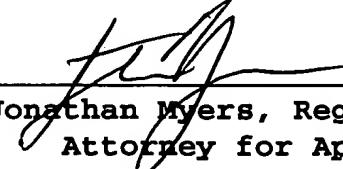
Appellant hereby requests that the Board of Appeals and Interferences refund Appellant's fee of \$250.00 paid on 29 September 2006 using form PTO 2038 to file a Notice of Appeal. Appellant encloses copies of the Notice of Appeal filed 29 September 2006 and form PTO 2038 as Exhibits A and A1. The reason for the request is as follows:

On 27 May 2005, Appellant had already filed a Notice of Appeal and already paid the fee of \$250.00 using form PTO 2038. Copies of both documents are enclosed as Exhibits B and B1. Appellant followed up on 19 July 2005 by filing an Appeal Brief Under 37 CFR 41.37 and paid the fee of \$250.00 again using form PTO 2038. A copy of page 1 of the appeal brief and a copy of the form

PTO 2038 are enclosed as Exhibits B2 and B3. Upon receiving Appellant's appeal brief the Examiner on 11 October 2005 reopened prosecution of this application and gave Appellants a new office action. In the new office action the Examiner indicated that should Appellant in the future initiate a new appeal, both the original payment of the notice of appeal and the original payment for filing the appeal brief may be applied to the new appeal. Appellant attaches a copy of the new office action as Exhibit C.

In view of the above Appellant respectfully requests a refund of \$250.00 which may be credited to the deposit account of the undersigned attorneys 18-2025.

Respectfully submitted,
THE FIRM OF KARL F. ROSS, PC



Jonathan Myers, Reg. No. 26,963
Attorney for Appellant

er
29 November 2006
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Cust. No.: 535
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Enclosures: Exhibit A - Notice of Appeal
Exhibit A1 - PTO 2038
Exhibit B - Notice of Appeal
Exhibit B1 - PTO 2038
Exhibit B2 - Appeal Brief (page 1)
Exhibit B3 - PTO 2038

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Exhibit A
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IN THE U.S. PATENT AND TRADEMARK OFFICE
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Inventor Gabor BOGYE

Patent App. 09/890,029

Filed 24 July 2001

Conf. No. 6045

For PHARMACEUTICAL COMBINATION OF PROGESTERONE AND
FOLIC ACID

Art Unit 1617 Examiner Hui, S

Hon. Commissioner of Patents
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NOTICE OF APPEAL

Applicant hereby appeals to the Board of Patent Appeals
and Interferences from the final decision dated 4 May 2006
rejecting claims 9-12, 20 and 25-39.

A response to the final rejection has been filed.

A PTO-2038 for \$250.0.00 for a small entity is enclosed.
If the PTO-2038 is missing or insufficient, please charge any
shortfall to account 18-2025 of the undersigned attorney firm.

Respectfully submitted,
The Firm of Karl F. Ross P.C.

by: Jonathan Myers, 26,963
Attorney for Applicant

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Application No. 09/890,029	Application No.	Serial No.	IDON Customer No.
Patent No. None	Patent No.	Registration No.	
Attorney Docket No. 21965		Mark	

Exhibit B

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IN THE U.S. PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF APPEALS AND INTERFERENCES

Inventor Gabor BOGYE

Patent App. 09/890,029

Filed 24 July 2001

Conf. No. 6045

FOR PHARMACEUTICAL COMBINATION OF PROGESTERONE AND
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Art Unit 1617

Examiner Hui, S

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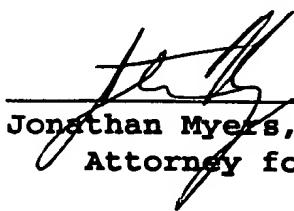
NOTICE OF APPEAL

Applicant hereby appeals to the Board of Patent Appeals and Interferences from the final decision dated 27 January 2005 rejecting claims 14.

A response to the final rejection has been filed.

A PTO-2038 for \$250.0.00 for a small entity is enclosed.
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Respectfully submitted,
The Firm of Karl F. Ross P.C.



Jonathan Myers, Reg. No. 26,963
Attorney for Applicant

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27 May 2005
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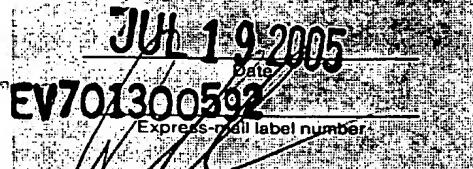
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Exhibit B2

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IN THE U.S. PATENT AND TRADEMARK OFFICE

Inventor Gabor BOGYE
 Patent App. 09/890,029
 Filed 24 July 2001 Conf. No. 6045
 For PHARMACEUTICAL COMBINATION OF PROGESTERONE AND FOLIC ACID
 Art Unit 1617 Examiner Hui, S
 Hon. Commissioner of PatentsAppealed 27 May 2005
 Box 1450
 Alexandria, VA 22313-1450

APPEAL BRIEF UNDER 37 CFR 41.37

Now comes appellant by his duly authorized attorney and submits his brief under the provisions of 37 CFR 41.37.

(i) REAL PARTY IN INTEREST

The real party in interest is the Appellant Gabor Bogye.

(ii) RELATED APPEALS AND INTERFERENCES

There are no prior or pending appeals, interferences or judicial proceedings known to Appellant or to the Appellant's legal representative, which may be related to, directly affect, or be directly affected by or have a bearing on the Board's decision in the pending appeal.

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Appeal Brief

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Application No. 09/890,029	Application No.	Serial No.	IDON Customer No.
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